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Cover photo: Two female scorpions, *Paruroctonus utahensis* (Vaejovidae), fighting on the desert floor in western Texas, USA. The animals were photographed at night, illuminated by UV light that caused their cuticles to fluoresce. (See page 54).
Photo by Douglas D. Gaffin.

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Revision and morphological phylogenetic analysis of the funnel web spider genus *Agelenopsis* (Araneae: Agelenidae)

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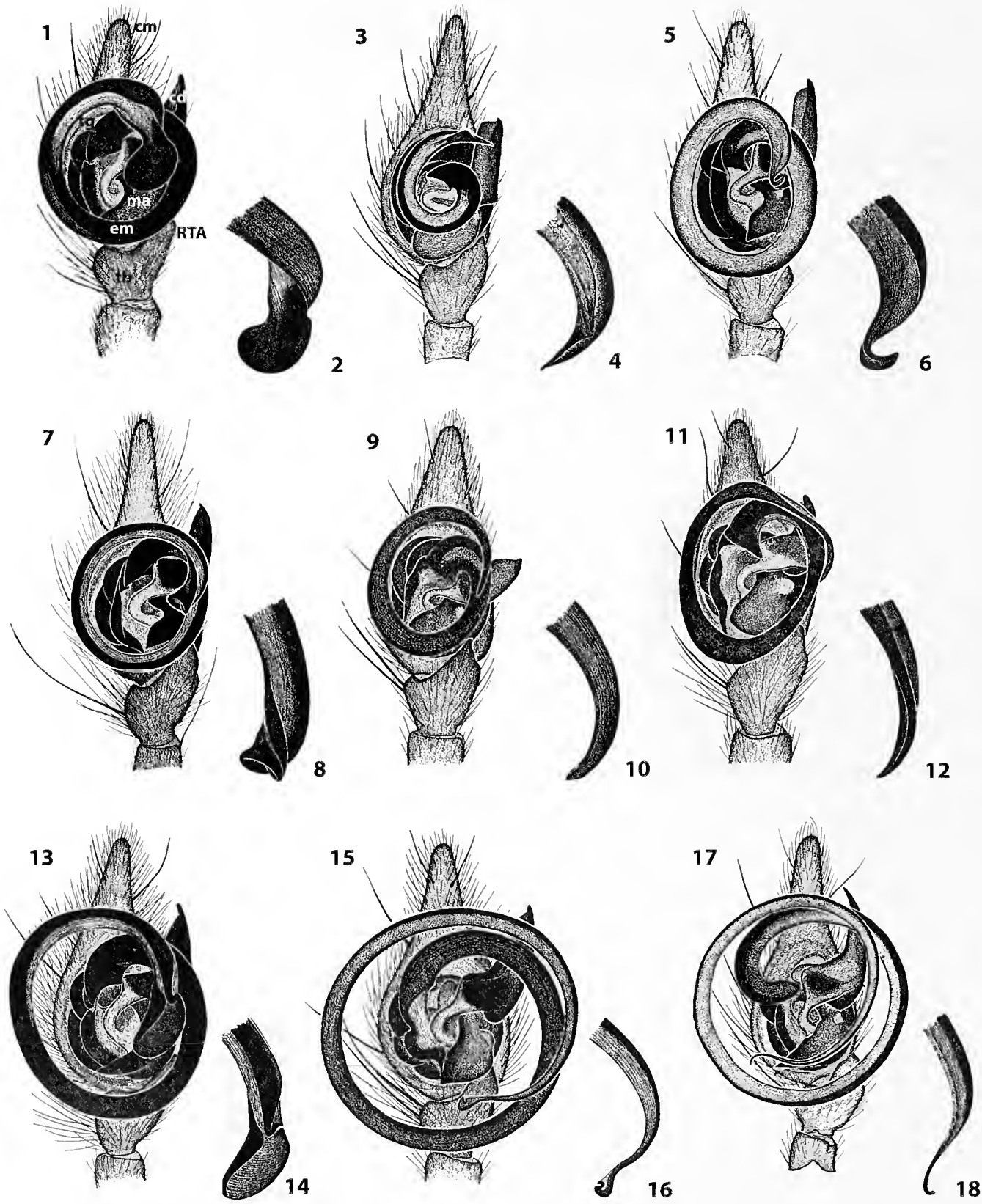
Abstract. The Nearctic agelenid spider genus *Agelenopsis* Giebel 1896 is revised, with redescriptions of the 13 known species including: *A. actuosa* (Gertsch & Ivie 1936), *A. aleenae* Chamberlin & Ivie 1935, *A. aperta* (Gertsch 1934), *A. emertoni* Chamberlin & Ivie 1935, *A. kastoni* Chamberlin & Ivie 1941, *A. longistyla* (Banks 1901), *A. naevia* (Walckenaer 1841), *A. oklahoma* (Gertsch 1936), *A. oregonensis* Chamberlin & Ivie 1935, *A. pennsylvanica* (C.L. Koch 1843), *A. potteri* (Blackwall 1846), *A. spatula* Chamberlin & Ivie 1935, and *A. utahana* (Chamberlin & Ivie 1933). We also include an identification key to the species and a species distribution map. Our cladistic analysis of *Agelenopsis* is based upon 31 genitalic and somatic characters using *Hololena hola* (Chamberlin 1928) as the outgroup taxon and including three species of *Barronopsis* Chamberlin & Ivie 1941 in the analysis since *Barronopsis* has been considered a sister taxon to *Agelenopsis* in previous work. The cladistic analysis found 22 most parsimonious trees unambiguously supporting *Agelenopsis* monophyly. The majority rule consensus provides support for a clade including (((*A. pennsylvanica* + *A. potteri*) + *A. actuosa*) + *A. emertoni*); another clade including ((((*A. aleenae* + *A. spatula*) + *A. aperta*) + *A. kastoni*) + *A. naevia*); and a third clade including ((*A. oregonensis* + *A. utahana*) + *A. longystyla*). Our analysis supports species groups proposed by researchers using molecular characters.

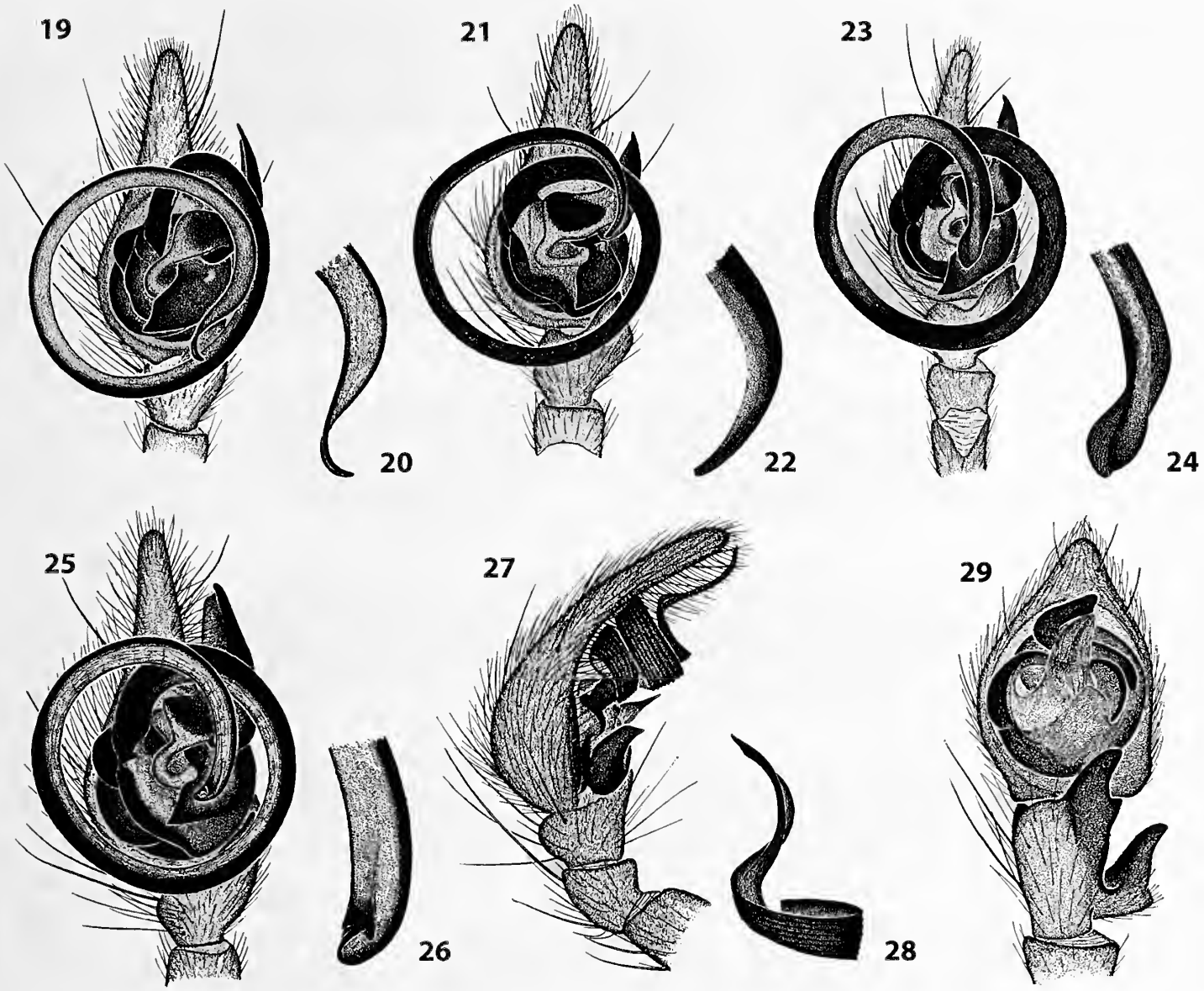
Keywords: Cladistic analysis, taxonomy, phylogeny, *Barronopsis*, *Hololena*

Since the formation of the family Agelenidae (Koch 1837), the relationships among and within genera have not been clearly defined and there is still need for much revision (Bennett & Ubick 2005). Agelenids are members of a family of funnel web spiders, including 300 species in North America, whose members all construct sheet webs with funnel retreats in various habitats, including grasses, among rocks or low bushes, and sometimes in protected places on buildings, wherever a substructure provides an adequate hiding area for the funnel portion of the web (Guarisco 2014 and pers. obs). The Nearctic genus *Agelenopsis* includes thirteen species that range in size from 6 to 18 mm: *Agelenopsis actuosa* (Gertsch & Ivie 1936), *A. aleenae* Chamberlin & Ivie 1935, *A. aperta* (Gertsch 1934), *A. emertoni* Chamberlin & Ivie 1935, *A. kastoni* Chamberlin & Ivie 1941, *A. longistyla* (Banks 1901), *A. naevia* (Walckenaer 1841), *A. oklahoma* (Gertsch 1936), *A. oregonensis* Chamberlin & Ivie 1935, *A. pennsylvanica* (C.L. Koch 1843), *A. potteri* (Blackwall 1846), *A. spatula* Chamberlin & Ivie 1935, and *A. utahana* (Chamberlin & Ivie 1933). While most agelenids have long posterior spinnerets, *Agelenopsis* is one of three genera in the family (*Agelenopsis*, *Calilena* Chamberlin & Ivie 1941 and *Melpomene* O. Pickard-Cambridge 1898) with the distal portion of the posterior spinnerets approximately twice the length of the basal segment (Bennett & Ubick 2005). All other genera have distal and basal segments approximately the same length. *Agelenopsis* males have a large, openly coiled embolus that lies flat across the face of the palp (Figs. 1–26). The female's epigynum is comprised of an open oval atrium with a coupling cavity that is clearly seen on the posterior edge of the atrium, separated by a sclerotized bridge (Figs. 30–42). Both the sweeping circular coil of the embolus and distinctive shape of the embolus tip in the male and presence of a coupling cavity in the female make *Agelenopsis* relatively straightforward to identify and distinguish from other agelenid genera. The taxonomic relationships among the species within the genus, however, are not so clearly defined. Nine of the 13 currently known species of *Agelenopsis* were originally placed within *Agelena*: *A.actuosa*, *A. aperta*, *A. longistyla*, *A. naevia*, *A. oklahoma*, *A. oregonensis*, *A. pennsylvanica*, *A. potteri*, and *A. utahana*. Through the first three decades of the twentieth century, collectors made various notes on and descriptions of spider species ultimately placed within the genus *Agelenopsis* (Petrunkevitch 1925; Gertsch 1934, 1936; Chamberlin & Ivie 1933, 1935; Gertsch & Ivie 1935, 1936; Exline 1938).

A revision of *Agelenopsis* and a few other genera in Agelenidae was published over seventy years ago (Chamberlin & Ivie 1941). Taxonomic contributions to this genus have since been made by a number of researchers (Seyler 1941; Chamberlin & Ivie 1944; Muma 1945; Gering 1953; Roth 1954, 1956; Roth & Braeme 1972; Roth & Brown 1986; Paison 1997; Ayoub et al. 2005; Stocks 2009; Galasso 2012). There have been a number of ecological and behavioral studies on *Agelenopsis*, predominantly on *A. aperta* (Riechert et al. 1973; Riechert 1974, 1976, 1978, 1981, 1982, 1985, 1986; Riechert & Tracy 1975; Gertsch & Riechert 1976; Riechert & Lockley 1984; Hammerstein & Riechert 1988; Riechert & Smith 1989; Riechert & Hedrick 1993; Singer & Riechert 1994; Riechert & Singer 1995; Galasso 2012; Guarisco 2014). Based on molecular phylogenetic work on the genus, *Agelenopsis* shares a sister relationship with *Barronopsis* (Ayoub et al. 2005; Stocks 2009). *Barronopsis*, originally a subgenus of *Agelenopsis* and elevated to its own genus by Lehtinen (1967), was included in our cladistic analysis. In the present paper we revise *Agelenopsis*, provide redescriptions of the 13 known species, provide detailed illustrations of male and female genitalic characters, include an identification key to the species and a species distribution map, and determine the monophyly



Figures 1–18.—Male palps in ventral view and detail of embolic tips for *Agelenopsis*. 1–2) *A. aleenae*, 3–4) *A. pennsylvanica* (note the subtriangular segment evident in the embolic tip of 4), 5–6) *A. potteri*, 7–8) *A. actuosa*, 9–10) *A. utahana*, 11–12) *A. oregonensis*, 13–14) *A. spatula*, 15–16) *A. longistyla*, 17–18) *A. oklahoma*. Abbreviations: cd = conductor, cm = cymbium, em = embolus, ma = median apophysis, RTA = retrolateral tibial apophysis, tb = palpal tibia, tg = tegulum. Drawings from Chamberlin & Ivie 1941, by permission from Entomological Society of America.

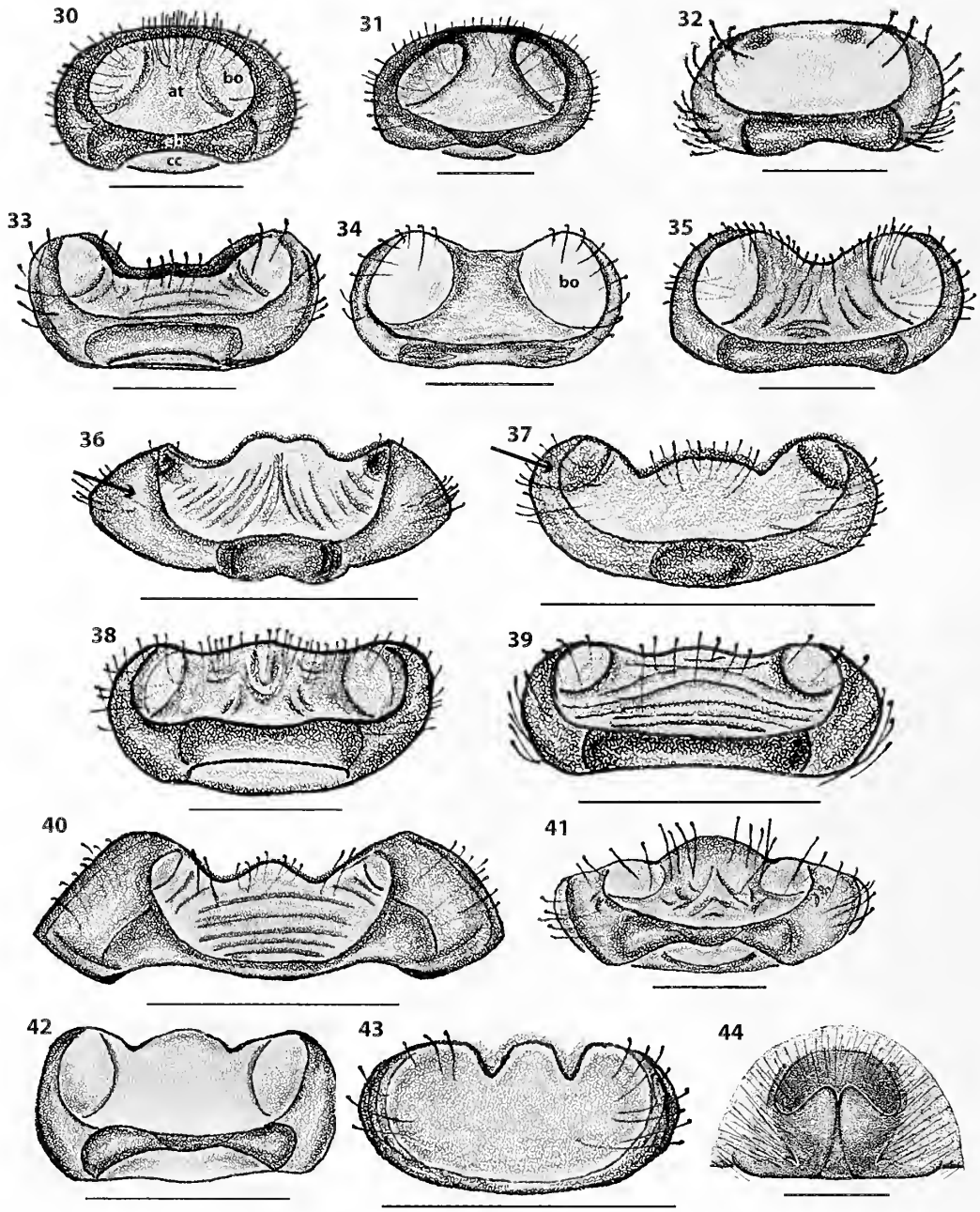


Figures 19–29.—Male palps in ventral view and detail of embolic tip for *Agelenopsis*, a representative *Barronopsis*, and the outgroup *Hololena hola*. 19–20) *A. kastoni*, 21–22) *A. naevia*, 23–24) *A. aperta*, 25–26) *A. emertoni*, 27–28) *B. floridensis*, 29) *H. hola* ventral pedipalp. All drawings from Chamberlin & Ivie (1941) except *H. hola* from Chamberlin & Gertsch (1928), by permission from Entomological Society of America.

of the genus as well as the relationships among the species within *Agelenopsis* using a morphological cladistic analysis.

METHODS

Material examined.—Specimens used were from the following institutions and private collections: American Museum of Natural History, New York (AMNH); New Mexico State University Arthropod Museum, Las Cruces (NMSU); Burke Museum of Natural History and Culture, University of Washington (BMNHC); California Academy of Sciences, San Francisco (CAS); Denver Museum of Nature & Science, Denver Colorado (DMNS); Florida State Collection of Arthropods, Gainesville, (FSCA); Hank Guarisco Collection, Lawrence, Kansas (HJG); Midwestern State University, Department of Biology, Wichita Falls, TX (MWSU); Museum of Comparative Zoology, Harvard University, Cambridge, MA (MCZ); National Museum of Natural History, Smithsonian Institute, Washington D.C. (USNM); Texas A&M, College Station, TX (TXAM); and University of Colorado, Boulder (CU). Vial identification numbers are included if the loaning institution provided them. We examined and photographed specimens using an Olympus SZX12 microscope with a mounted Olympus UCMD3 camera and Spot Basic™ (4.1) software. We used the Helicon Focus stacking software and Adobe Photoshop CS5 for final images and ArcGIS version 10.0 to prepare distribution maps. Many of the labels for the older specimens used in this study only list degrees or degrees and minutes for the coordinates; some provide no locality data at all. Since so many of the specimens examined for this revision were poorly georeferenced, for the distribution map we supplemented these data with data from specimens borrowed from Ayoub and Riechert for their study (Ayoub & Riechert 2004) and from data from several collections accessed from the Symbiota Collections of



Figures 30–44.—Epigyna in ventral view of *Agelenopsis*, *Barronopsis*, and *Hololena*. 30) *A. pennsylvanica*, 31) *A. potteri*, 32) *A. actuosa*, 33) *A. naevia*, 34) *A. aleenae*, 35) *A. spatula*, 36) *A. utahana*, 37) *A. oregonensis*, 38) *A. oklahoma*, 39) *A. kastoni*, 40) *A. longistyla*, 41) *A. aperta*, 42) *A. emertoni*, 43) *B. floridensis*, 44) *H. hola*. Scale bars = 0.5 mm. Abbreviations: at = atrium, bo = opening to bursa, cc = coupling cavity, eb = epigynal bridge. Drawing of *H. hola* from Chamberlin & Gertsch (1928) by permission from Entomological Society of America.

Arthropods Network database (http://symbiota4.acis.ufl.edu/scan/portal/). Under Taxonomy, we recorded locality data as written on the label to accurately reflect those data as recorded, but have converted all data to decimal degrees where possible for consistency in presentation. Only those specimens for which good locality information was discernible were used for distribution maps unless they represent unique state records, in which case these were also included on the map.

Measurements.—We generated our own set of measurements based upon characteristics considered diagnostic by Chamberlin & Ivie (1941), Paison (1997), and Stocks (2009). All meristic and genitalic measurements were documented in millimeters (mm) using an Olympus SZX12 microscope and Spot Basic™ (4.1) software. For both males and females, we determined the length of femur and tibia-patella for legs I and IV, cephalothorax length, cephalothorax width both at the narrowest point just behind the eyes and at the widest point, body length (excluding spinnerets), and length of basal and distal segments of the posterior lateral spinnerets. Both leg and spinneret measurements were taken from a lateral view on the left side of the specimen when possible (otherwise, from the right side). Cephalothorax and body length measurements were assessed from a dorsal perspective. Additionally, we took a number of epigynal measurements: atrial length (at widest and narrowest points) and width, epigynal length and width, epigynal bridge length and width (epigynal bridge labeled "eb"

Table 1.—Character matrix for the cladistic analysis of the spider genus *Agelenopsis*. See the text for a description of the 31 characters used. *Hololena hola* was used as the outgroup. Three species of *Barronopsis* were included because other studies have suggested *Barronopsis* is a sister taxon to *Agelenopsis*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
H. hola	0	0	0	0	0	0	?	?	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. floridensis	0	1	1	1	0	2	0	0	1	0	1	0	2	?	0	1	0	0	2	1	0	1	1	2	1	1	0	0	1	2	2
B. jeffersi	0	1	1	1	0	2	0	0	1	0	1	0	2	?	0	1	0	0	2	1	0	1	1	2	1	1	0	1	1	2	1
B. texana	0	1	1	1	0	2	0	0	1	0	1	0	2	?	0	1	0	0	2	1	0	1	1	2	1	1	0	0	1	2	1
A. actuosa	1	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	1	1	0	0	1	1	0	1	1	0	0	0	0	1	2
A. aleenae	1	0	0	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1	0	1	0	0	0	0	2	1	0
A. aperta	1	0	0	1	1	1	1	1	1	0	0	1	2	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	2	1	0
A. emertoni	1	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	1	1	0	1	0	0	0	1	0	0	0	0	1	0
A. kastoni	1	0	0	0	1	1	1	2	1	0	1	1	2	1	1	1	1	1	1	0	1	1	0	0	0	0	1	0	1	1	0
A. longistyla	1	0	0	0	0	1	1	0	1	0	0	0	2	1	0	1	1	1	1	0	1	1	0	0	0	0	0	1	1	2	1
A. naevia	1	0	0	0	1	2	1	1	0	0	0	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	1	0	1
A. oklahoma	1	0	0	0	0	0	1	0	1	0	0	1	2	1	1	1	1	1	1	0	1	1	0	0	0	0	1	0	1	2	1
A. oregonensis	1	0	0	0	0	1	0	0	2	0	1	0	2	0	1	1	1	1	1	0	1	1	0	1	0	0	0	0	1	1	1
A. pennsylvanica	1	0	0	1	1	0	1	0	1	0	0	1	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	1	0	2
A. potteri	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0	1	0	1	0	2
A. spatula	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	0	2	0	0
A. utahana	1	0	0	0	0	2	1	2	2	1	1	0	2	0	0	1	1	1	0	0	1	0	0	1	0	0	0	0	1	0	1

in Fig. 30), width of opening of the bursa from its outer edge (as visible externally) to atrial edge (i.e. the width of the opening labeled "bo" in Figs. 30, 34), and coupling cavity length and width (coupling cavity is labeled "cc" in Fig. 30).

Cladistic analysis.—The cladistic analysis was based on the 13 ingroup taxa with *Hololena hola* (Chamberlin 1928) (from Chamberlin & Gertsch 1928) used as an outgroup (Figs. 29, 44, 60). Because other studies suggest that *Barronopsis* is a sister taxon to *Agelenopsis* (Ayoub et al. 2005; Stocks 2009) and because this genus was originally described as a subgenus of *Agelenopsis* (Giebel 1869), we included three species in this genus in our cladistic analysis: *Barronopsis floridensis* (Roth 1954), *B. jeffersi* (Muma 1945), and *B. texana* (Gertsch 1934). Our final character matrix included 31 morphological characters listed below as well as the ratios determined to be statistically significant based upon one-way ANOVA analyses. The final character matrix is presented in Table 1. The cladistic analysis was run using PAUPRat on the CIPRES Science Gateway (Nixon 1999; Sikes & Lewis 2001; Miller et al. 2010). Our optimality criterion was parsimony with heuristic search criteria, all characters were weighted equally, and we used tree bisection reconnection (TBR) branch swapping with random addition. We recorded the following ratios: cephalothorax width at widest point/cephalothorax length, cephalothorax length/body length (excluding spinnerets), and atrial width/atrial length, but ultimately eliminated the first two from the matrix because they were parsimony uninformative. The atrial width/atrial length character was statistically significant based upon a one-way ANOVA analysis (Fcrit = 1.78, df = 12, $P < 0.001$). For this character, we determined character states using mean ± variance to find natural groupings. For example, we found the species to fall into groups in which the mean + var was < 0.38; between 0.38–0.46; or > 0.46.

RESULTS

Morphological characters.—The morphological characters used in the cladistic analysis included 31 characters as described below.

Female Characters:

1. Coupling cavity: [0] absent (e.g., Figs. 43, 44), [1] present (e.g., Fig. 30)
2. Bursa torsion: [0] loosely or not twisted distally (e.g., Figs. 45, 60), [1] tightly twisted distally (e.g., Fig. 59)
3. Number of turns of fertilization ducts visible at base of bursa: [0] ≤ 2 turns (e.g., Fig. 47), [1] 3 turns (e.g., Fig. 59)
4. Distance between bursae at base: [0] ≥ 1.5 × diameter of bursa (e.g., Figs. 49, 60), [1] < 1.5× diameter of bursa (e.g., Figs. 45, 59)
5. Spermathecae: [0] not touching (e.g., Figs. 52, 55), [1] touching (e.g., Figs. 49, 56)
6. Orientation of spermathecae in relation to bursae: [0] dorsal of bursae (e.g., Figs. 47, 53), [1] centered between bursae (e.g., Figs. 54, 55), [2] ventral of bursae (e.g., Figs. 48, 51)
7. Orientation of connecting tube as it enters spermatheca: [0] lateral (e.g., Fig. 50), [1] ventral (e.g., Fig. 49)
8. Connecting tube pattern as it enters spermatheca: [0] straight entry (e.g., Fig. 53), [1] tubes diverge and converge again (e.g., Figs. 49, 50), [2] complex looping (e.g., Fig. 54)
9. Shape of spermatheca: [0] spherical (e.g., Figs. 48, 58), [1] oblong (e.g., Figs. 47, 49), [2] tube-like (e.g., Figs. 51, 52)
10. Orientation of spermatheca: [0] longitudinal (e.g., Figs. 49, 50), [1] transverse (Fig. 51)
11. Shape of diverticle: [0] curved and tubular (e.g., Fig. 57), [1] bulbous (e.g., Figs. 51, 52)
12. Distance between diverticles: [0] > ½ diameter of diverticle (e.g., Fig. 50), [1] < ½ diameter of diverticle (e.g., Fig. 57)
13. Anterior atrial edge: [0] smooth (e.g., Figs. 30, 32), [1] monolobed (e.g., Figs. 33, 34), [2] strongly or moderately bilobed (e.g., Figs. 37, 38)
14. If epigynal bridge present: [0] ≤ half the diameter of atrium (e.g., Figs. 31, 33), [1] > than half the diameter of atrium (e.g., Figs. 30, 40)

15. Copulatory duct opening of atrium: [0] partially or not visible (e.g., Figs. 36, 40), [1] mostly visible (e.g., Figs. 34, 37)

Male characters:

16. Complexity of RTA: [0] complex (Fig. 29), [1] simple (e.g., Figs. 1, 3)
17. Shape of RTA: [0] truncate (e.g., Figs. 9, 29), [1] pointed (e.g., Figs. 1, 23)
18. Orientation of median apophysis (directed): [0] up, parallel with cymbium tip (e.g., Figs. 27, 28), [1] down, at a 45° angle from cymbium base (e.g., Figs. 1, 5)
19. Shape of median apophysis: [0] thick, more rounded tip (e.g., Figs. 7, 9), [1] thick, somewhat pointed (e.g., Figs. 1, 23), [2] sharp, thorn-like spur (e.g., Fig. 27)
20. Appearance of tegulum: [0] remains an integral part of embolic structure with either no or slightly rounded apophysis (e.g., Figs. 15, 29), [1] projects from embolic structure ending in a pointed apophysis (e.g., Fig. 27)
21. Conductor size: [0] small and short (e.g., Figs. 27, 29), [1] large and long (e.g., Figs. 1, 3)
22. Conductor shape: [0] truncate (e.g., Figs. 3, 25), [1] pointed (e.g., Figs 1, 7)
23. Surface texture of embolus: [0] lamellate throughout (e.g., Figs. 13, 15), [1] smooth proximally, lamellate distally (e.g., Fig. 27)
24. Coiling of embolus: [0] loose throughout (e.g., Figs. 23, 25), [1] slightly tight throughout (e.g., Figs. 1, 7), [2] very tight basally and loose distally (e.g., Fig. 27)
25. Embolic subtriangular segment: [0] absent (e.g., Figs. 2, 24), [1] present (e.g., Figs. 4, 26)
26. Embolus orientation in relation to plane of the cymbium: [0] parallel (e.g., Figs. 3, 11), [1] oblique (e.g., Fig. 27)
27. Embolic tip direction: [0] not recurved (e.g., Figs. 16, 26), [1] recurved (e.g., Figs. 18, 20)
28. Embolic tip detail: [0] without notch (e.g., Figs. 2, 4), [1] with notch (e.g., Fig. 16)
29. Embolic tip shape: [0] remains thick throughout (e.g., Figs. 8, 26), [1] tapers distally (e.g., Figs. 16, 20), [2] thickens distally (spatulate) (Figs. 2, 14, 24)
30. Embolic tip termination angle: [0] $\leq 470°$ (e.g., Figs. 4, 22), [1] 540° (e.g., Figs. 2, 8), [2] $\geq 720°$ (e.g., Figs. 16, 18)

Meristic character:

31. Atrium length/atrium width (the ratio reflects shape of atrium, be it more oblong or openly rounded): [0] > 0.38 and < 0.46 (e.g., Figs. 34, 41), [1] < 0.38 (e.g., Figs. 33, 40), [2] > 0.46 (e.g., Figs. 30, 31)

Cladistic analysis.—Twenty-nine of the characters were parsimony informative. The heuristic search resulted in 22 best fit trees retained in memory each with tree length = 87, consistency index (CI) = 0.46, homoplasy index (HI) = 0.54, retention index (RI) = 0.63, and rescaled consistency index (RC) = 0.29. The 50% majority rule consensus tree (Fig. 62) supported the monophyly of *Agelenopsis*. It also provided support for a clade including (((*A. pennsylvanica* + *A. potteri*) + *A. actuosa*) + *A. emertoni*); another clade including ((((*A. aleenae* + *A. spatula*) + *A. aperta*) + *A. kastoni*) + *A. naevia*); and a third clade including ((*A. oregonensis* + *A. utahana*) + *A. longystyla*). *Agelenopsis oklahoma* was on a separate clade more distantly related to the clade including *A. longistyla, A. oregonensis,* and *A. utahana* than to the other species.

KEY TO SPECIES OF *AGELENOPSIS*

1(a) Males 2
1(b) Females 14
2(a) Diameter of coiled embolus not substantially wider than width of cymbium (Figs. 1, 3) 3
2(b) Diameter of coiled embolus noticeably wider than width of cymbium (Figs. 13, 15, 17) 8
3(a) Embolic tip spatulate (Fig. 2) ..... ***aleenae***
3(b) Embolic tip pointed (Fig. 4) 4
4(a) Embolus coil makes full circle with tip position perpendicular to cymbium (Fig. 3) ..... ***pennsylvanica***
4(b) Embolus coil makes more than a full circle (Figs. 5, 7) 5
5(a) Embolic tip recurved (Fig. 6) ..... ***potteri***
5(b) Embolic tip procurved or generally straight (Figs. 8, 10) 6
6(a) Embolic tip generally straight and twisted at tip (Fig. 8) ..... ***actuosa***
6(b) Embolic tip procurved and tapered (Figs. 10, 12) 7
7(a) Conductor truncated (Fig. 9) ..... ***utahana***
7(b) Conductor pointed (Fig. 11, pointed tip seen behind embolus) ..... ***oregonensis***
8(a) Embolic tip spatulate (Fig. 14) ..... ***spatula***
8(b) Embolic tip other than spatulate (Figs. 16, 18) 9
9(a) Embolic tip hooked (Fig. 16) ..... ***longistyla***
9(b) Embolic tip other than hooked (Figs. 18, 20, 22) 10
10(a) Embolus coils make 2 full circles (Fig. 17) ..... ***oklahoma***
10(b) Embolus coils make less than 2 full circles (Fig. 19, 21) 11
11(a) Embolic tip recurved (Fig. 20) ..... ***kastoni***
11(b) Embolic tip procurved (Figs. 22, 24, 26) 12
12(a) Embolic tip procurved and tapering (Fig. 22) ..... ***naevia***
12(b) Embolic tip procurved and twisted (Figs. 24, 26) 13
13(a) Conductor tip pointed (Fig. 23) ..... ***aperta***
13(b) Conductor tip claw-like (Fig. 25) ..... ***emertoni***
14(a) Atrial opening generally rounded; width less than twice its length (Figs. 30, 31) 15

14(b) Atrial opening more distinctly oval than rounded; width at least twice the length (Figs. 32, 33, 35) 16
15(a) Epigynal bridge almost as wide as atrial opening (Fig. 30) ..... ***pennsylvanica***
15(b) Epigynal bridge much shorter than atrial opening (Fig. 31) ..... ***potteri***
16(a) Anterior edge of atrial opening generally forms a smooth arc; epigynal bridge pinched in the middle, thick and wide, copulatory duct openings not visible (Fig. 32); internally, tip of bursa strongly procurved (Fig. 47) ..... ***actuosa***
16(b) Anterior edge of atrial opening does not form a smooth arc (Figs. 33, 34) 17
17(a) Anterior edge of atrial opening is convex at center (Figs. 33, 34, 35) 18
17(b) Anterior edge of atrial opening is concave at center (Figs. 36, 37, 38) 20
18(a) Squared off, single inverted lobe at center of anterior edge of atrial opening; epigynal bridge short and thick (Fig. 33); spermatheca sits well back ventrally (Fig. 48) ..... ***naevia***
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19(a) Bursa openings rounded, closely set, and very visible (Fig. 34); conducting tube emerges from top of bursa to descend to spermatheca (Fig. 49, ct) ..... ***aleenae***
19(b) Bursa openings widely set and only partially visible (Fig. 35); thickened diverticle visible behind widely set bursae (Fig. 50, dt) ..... ***spatula***
20(a) Epigynal bridge short and thick (Figs. 36, 37) 21
20(b) Epigynal bridge not short and thick (Figs. 38, 39) 22
21(a) Sclerotized edges of epigynum broaden anteriorly (Fig. 36, arrow) ..... ***utahana***
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22(a) Atrial opening about 4 times as wide as long, almost capsule-like (Figs. 38, 39) 23
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23(a) Copulatory tube extends from top of bursa to spermatheca (Fig. 53, ct) ..... ***oklahoma***
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24(a) Width of sclerotized lateral edge of epigynum half the width of entire atrium (Fig. 40); very wide-set bursae basally (Fig. 55) ..... ***longistyla***
24(b) Width of sclerotized lateral edge of epigynum less than half the width of entire atrium (Figs. 41, 42) 25
25(a) Diameter of atrium wider in center than on sides; sclerotized edges of epigynum broaden rather than taper anteriorly; epigynal bridge extends under cuticle creating distinct demarcation (Fig. 41) ..... ***aperta***
25(b) Diameter of atrium is about as wide in center as on sides; sclerotized edges of epigynum taper rather than broaden anteriorly; epigynal bridge does not extend under cuticle (Fig. 42) ..... ***emertoni***

TAXONOMY

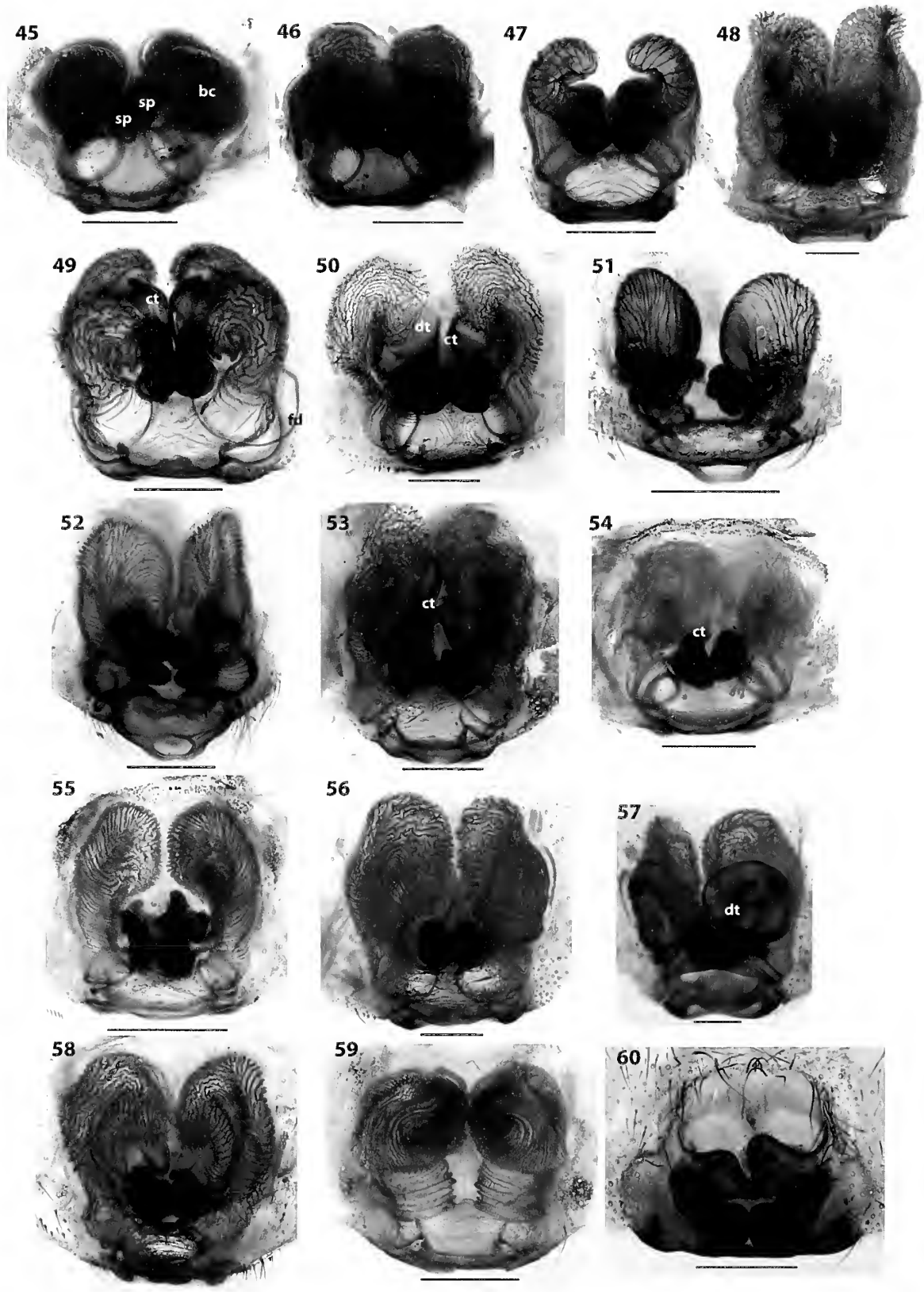
Family Agelenidae C.L. Koch 1837
Genus *Agelenopsis* Giebel 1869

Type Species.—*Agelena potteri* Blackwall 1846: 43.

Diagnosis.—*Agelenopsis* is distinguished from other agelenid genera by the following combination of characteristics: 1) conspicuously long PLS with distal segment usually twice the length of basal; 2) looping, circular embolus, lamellate throughout (Figs. 1–25); 3) distinctly large and long conductor but reduced tegular apophysis and tegulum (Figs. 1–25); 4) presence of coupling cavity on posterior edge of epigynum resulting in an epigynal bridge on the posterior edge of the atrium (Figs. 30–42); 5) two distinct loopings of the fertilization duct around the base of the bursa (Figs. 45–58); 6) plicated bursa generally inflated and erect throughout (Figs. 45–58).

Agelenopsis can be distinguished from *Barronopsis* by the presence of a coupling cavity in *Agelenopsis* (compare Figs. 42, 43). Furthermore, the median apophysis (ma) in *Barronopsis* tapers to a thin, elongated point (Fig. 27) whereas the ma in *Agelenopsis* is blunter at the tip. The conductors of both genera also vary. That of *Agelenopsis* is large and is either pointed or truncate at its distal end while that of *Barronopsis* is small in comparison and is uniformly pointed. *Agelenopsis* and *Barronopsis* are unique in Agelenidae for having large, coiling emboli. The males of the remaining genera have short, curving compact emboli, with the exception of *Tortolena* whose embolic structure, while compact, forms a figure–8 (Bennett & Ubick 2005). The primary difference between the emboli of *Agelenopsis* and *Barronopsis* lies in the coiling and tip structure. The *Barronopsis* embolus is oriented obliquely to the plane of the cymbium, is smooth proximally, lamellate distally with multiple, tight coils basally that loosen distally (Figs. 27, 28). Its tip tapers distally and is consistently notched. In *Agelenopsis*, the embolus is lamellate throughout and has one to one and a half slightly tight or loose coils that run parallel to the plane of the cymbium. Its tip may be notched or not, may taper distally or not, but usually has distinctive tips that make it easy to identify to species (Figs. 1–26).

Description of the genus.—General morphological characteristics as for the family. Body length varies throughout genus, ranging from 4–20 mm. Carapace uniformly longer than wide, squared-off at anterior end, broadening and rounded through thoracic region. Cephalothorax coloration ranges from reddish-yellow to reddish-brown with two thin brown bands widening as they extend posteriorly. Fovea is longitudinally oriented. A fine layer of plumose hairs covers the cephalothorax, abdomen, and legs. Eight small eyes are arranged in two strongly procurved rows. Clypeus is approximately two times diameter of AME. Endites, reddish-brown with lighter edges, are somewhat convergent at anterior edge and about two times the length of labium which is generally as long as wide. Sternum is reddish-brown in color and slightly longer than wide. Chelicerae are long and robust



Figures 45–60.—Dorsal view of dissected female genitalia for *Agelenopsis* and *Barronopsis* and ventral view of *A. aperta* and *H. hola* epigyna. 45) *A. pennsylvanica*, 46) *A. potteri*, 47) *A. actuosa*, 48) *A. naevia*, 49) *A. aleenae*, 50) *A. spatula*, 51) *A. utahana*, 52) *A. oregonensis*, 53) *A. oklahoma*, 54) *A. kastoni*, 55) *A. longistyla*, 56) *A. aperta*, 57) ventral view of dissected genitalia of *A. aperta* showing the diverticle (dt), 58) *A. emertoni*, 59) *B. floridensis*, 60) ventral view of *H. hola* showing bursae lying below the atrium. Scale bars = 0.5 mm. Abbrevations: bc = bursa copulatrix, ct = conducting tube, dt = diverticle, fd = fertilization duct, sp = spermatheca.

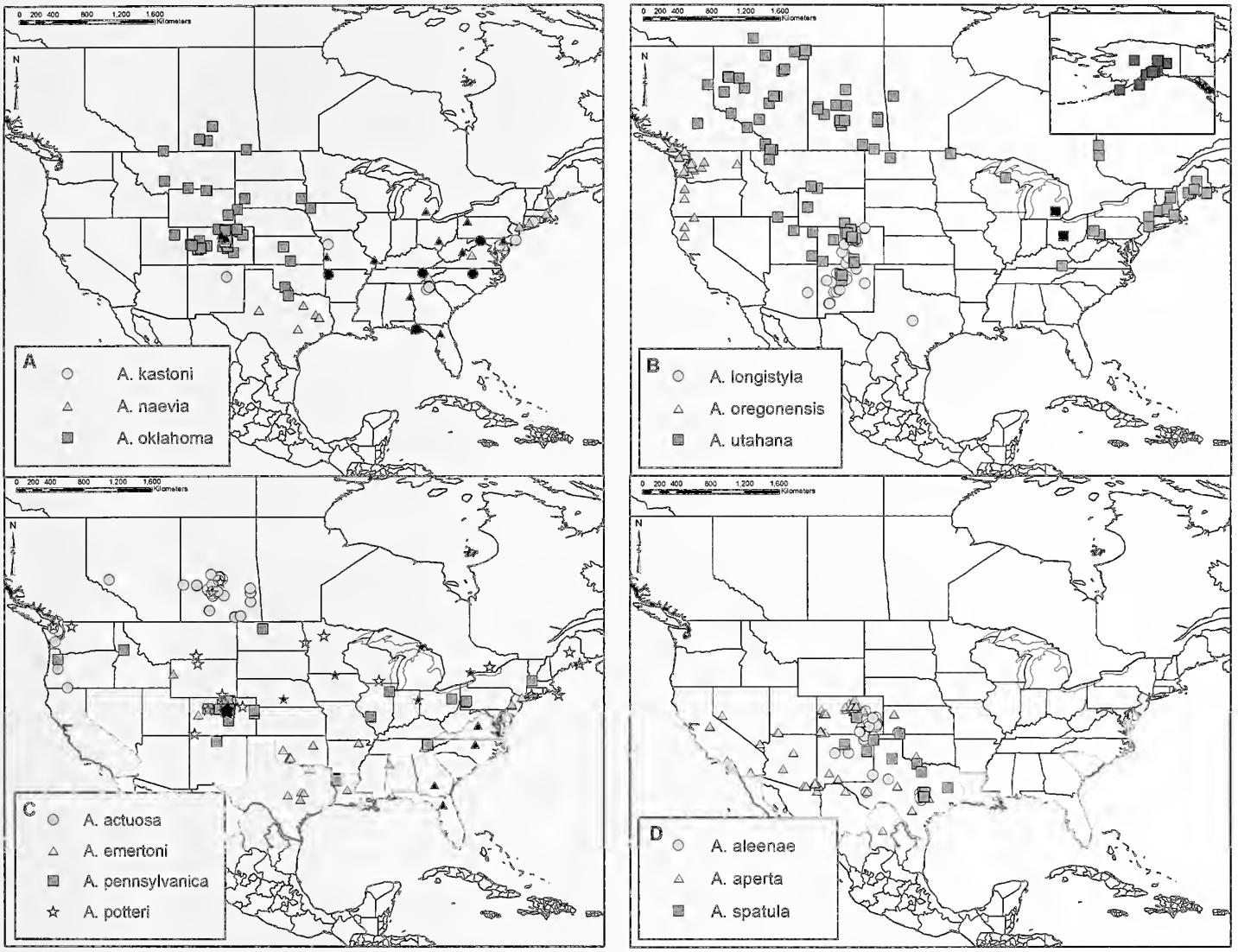


Figure 61.—Distribution map of *Agelenopsis* species. A) Distribution of *A. kastoni, A. naevia*, and *A. oklahoma*; B) Distribution of *A. longistyla*, *A. oregonensis*, and *A. utahana*; C) Distribution of *A. actuosa*, *A. emertoni*. *A. pennsylvanica*, and *A. potteri*; D) Distribution of *A. aleenae*, *A. aperta*, and *A. spatula*. Solid black symbols on the maps represent records of those species for states for which we have only state or county records and no other locale information, e.g., there is one record of *A. kastoni* in Anderson County, Tennessee.

and a deep brownish-red color. Each chelicera has a boss, 2–4 retromarginal teeth, and 3–4 promarginal teeth. Coloration of abdomen is highly variable from gray to brown, broken with two lighter, longitudinal bands of solid stripes or chevrons, regardless of size. Freckling is sometimes present dorso-posteriorly. Female abdomen is larger and more rounded than that of male which is more oblong in shape. Leg coloration is highly variable, but always banded. Legs are generally robust, with I and IV being longer than II and III. Leg pattern by length: IV, I, II, III. Setae and microsetae appear in pairs or sets of three on the femur, patella, tibia, metatarsus, and tarsus, becoming increasingly shorter and more profuse moving distally. Trichobothria on tarsi are of varying length. Spinnerets are uniformly yellowish-brown with the distal segment of the spinnerets being almost always longer than the basal. Anterior spinnerets are truncated while posterior spinnerets are long and tapering towards the distal end.

The epigynal atrium of females is uniformly transversely rounded and undivided with a sclerotized rim (Figs. 30–42). In *Agelenopsis*, variation in shape of anterior edge of atrium helps distinguish one species from another and distinguishes it from the closely related *Barronopsis* in which the anterior sclerotized margin has tooth-like invaginations into the atrium (compare Figs. 30–42 with Fig. 43). Openings to the bursa (bo in Fig. 30) within the margins of the atrium are either easily discernible or indistinct. Copulatory ducts and bursae are not visible externally, but a shadowing of the spermathecae is sometimes noticeable on the ventral surface above the epigynum (although not in all specimens). Internally, fertilization ducts spiral around the base of the bursae, winding up to spermathecae (Figs. 45–58). The internal structure of the female genitalia is distinct from the bursa and copulatory ducts in *Barronopsis* (compare Figs. 45–58 to Fig. 59). The bursae of *Agelenopsis* are generally more inflated and loose than those of *Barronopsis* which are characteristically tightly

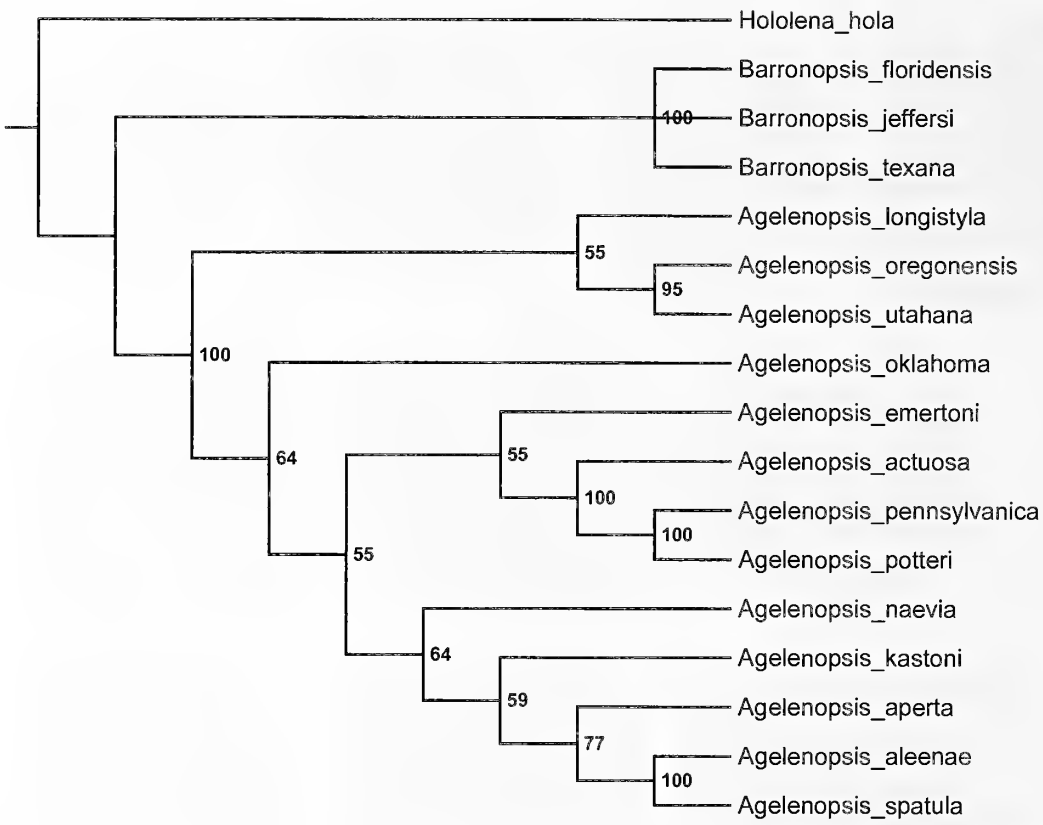


Figure 62.—50% majority rule consensus tree of the 22 minimal length trees of 87 steps found by PAUPRat when analyzing the character matrix presented in Table 1 (Length = 87, Consistency Index = 0.46, Homoplasy Index = 0.54, Retention Index = 0.63, Rescaled Consistency Index = 0.29). Numbers above clades are the bootstrap support values > 50% for those clades.

twisted (Fig. 59). The fertilization ducts wind three times around the base of the bursa in *Barronopsis* and only two times in *Agelenopsis*. Coupling cavity is wider than long and sits at a 90° angle below the atrium (Fig. 30, labeled "cc").

Habitat.—All thirteen species of *Agelenopsis* prove to be opportunistic both in habitat choice and web design. While these spiders tend to build their webs in both short and tall grass prairie in rural areas, they will construct webs in whatever setting, be it natural or manmade, that affords them the most protection, although Guarisco (2014) suggests that sympatric species are segregated by habitat. In our observations on collection trips in northeastern New Mexico and southeastern Colorado where we collected primarily *A. aleenae*, web design did not vary much by location or habitat and was only limited in size by the amount of space available to build a web. A typical web took the form of a single, horizontal sheet, conforming in shape to its surrounding support structure, and reinforced with multiple strands of silk above the sheet (Fig. 63). Each web had one, and often multiple funnels, some of which remained distinctly separate while others converged below the sheet construction (pers. obs.). An earlier study examined the correlation between web size and hunger (Riechert 1981, 1982), but we noted web size to be predominantly dependent on the size of the web's support structure. In our observations, most of the time, webs were built close to the ground, usually at approximately 10 cm. The only exceptions were those built high off the ground in very sturdy structures with impassable, protective retreat areas like grooved tree limbs or open pipes where the spiders were secure from disturbance from cattle, wind, predators, and water (either in the form of rain or flooding), or those built at ground level, either within lava beds or leaf litter. In a study on web-site selection, Riechert & Tracy (1975) and Riechert (1976) rated leaf litter as a very important factor in web placement, even higher than prey availability, because such a substrate provides the funnels and burrows with a constant temperature. The areas where we collected the most spiders had the highest densities of vegetative litter.

Agelenopsis actuosa (Gertsch & Ivie 1936)
Agelena actuosa Gertsch & Ivie 1936: 22, f. 46–47.
Figs. 7, 8, 32, 47, 61C

Type specimens.—Male holotype examined: *Oregon*, Tillamook County, Cape Meares, 22 August 1931, R.W. Macy, AMNH. Paratypes examined: Male paratype from Oregon, Benton County, Corvallis, 16 August 1934, J. Schuh, AMNH. Two male paratypes from St. Thomas, Ontario, Fall, 1929, L.E. James, AMNH.

Etymology.—None provided but "*actuosa*," in Latin means "full of activity," an appropriate descriptive adjective for any fast-moving agelenid.

Diagnosis.—The *A. actuosa* male is distinguished from other species in the genus by its generally straight-edged embolic tip (Figs. 7, 8), the distal edge of which twists over on itself from one edge to the other (Fig. 8). The *A. actuosa* female is

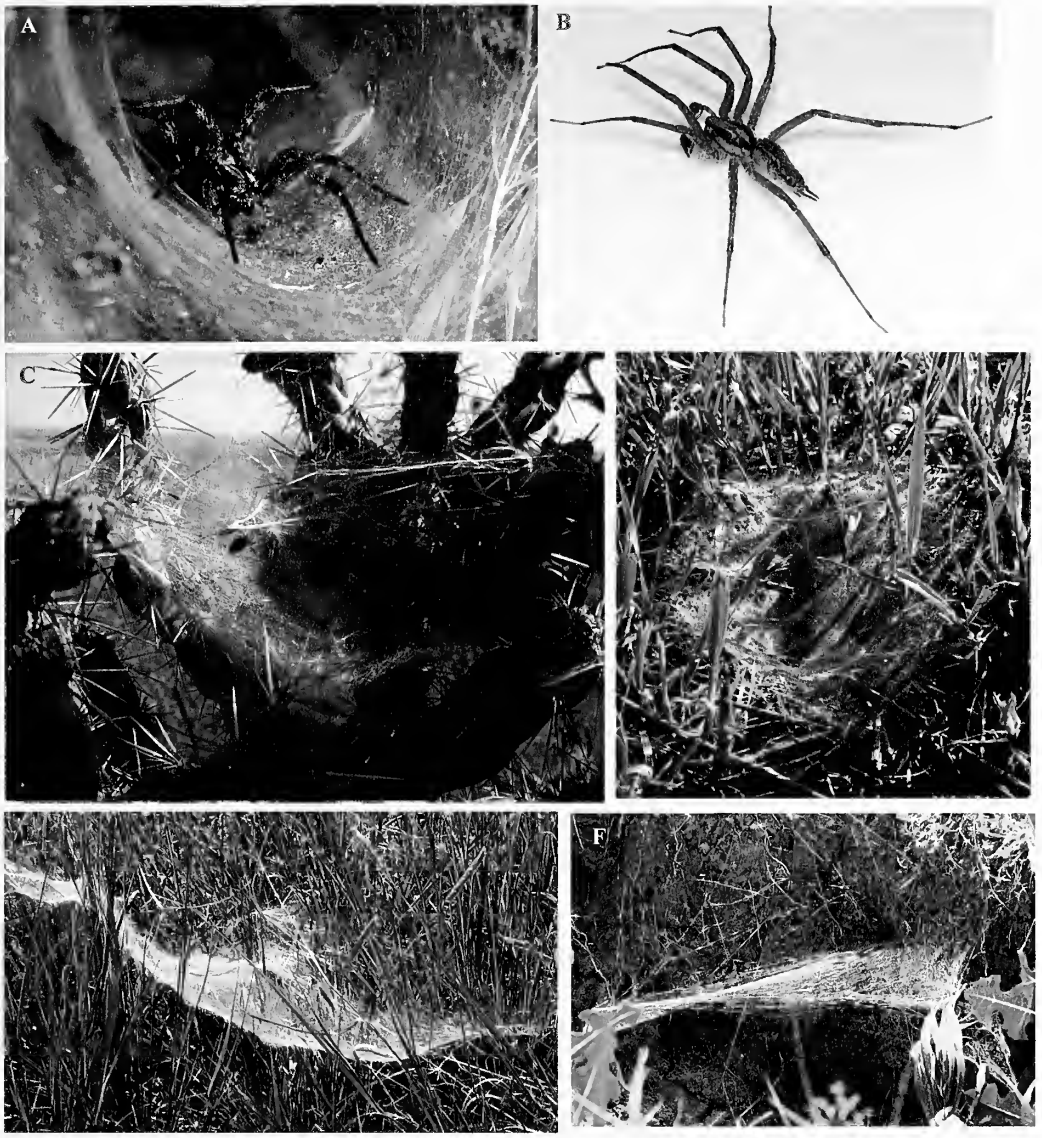


Figure 63.—*Agelenopsis* spiders and diversity of web architecture. A) Juvenile *Agelenopsis* in its funnel; B) *Agelenopsis aperta* male showing the long spinnerets; C) Web with funnel in branches of a cholla cactus; D) Web with funnel in the soil and the sheet web expanded on the surface; E) Lateral view of web showing expanse of the sheet up in the grass; F) Funnel built in a soil bank. Photos A, B, and F by Buzz Morrison, used with permission. Photos C, D, and E by JWZ.

distinctive in its uniquely shaped bursae, each curving sharply laterally toward the other (Fig. 47).

Description.—General description as for the genus. Male (n = 32): overall length 6.78–11.30 mm; carapace width at its widest point 2.14–4.01 mm; carapace width at its narrowest point 1.40–1.78 mm. Shape of retrolateral tibial apophysis (RTA) is nipple-like; shape of median apophysis is thick and somewhat rounded; conductor size is large with generally pointed shape; coiling of embolus is tight with an embolic tip termination angle of 540°; embolus subtriangular segment is present; embolic tip orientation is not recurved; embolic tip is twisted; anneli on subtegulum are present (Figs. 7, 8). Female (n = 34): overall length 9.50–14.90 mm; carapace width at its widest point 2.69–4.23 mm; carapace width at its narrowest point 1.55–2.39 mm. Bursa is generally longer than wide, leaning ventrally when viewed from above anterior edge, and widely spaced from other bursa at the tip, but not basally; orientation of each bursa tip is strongly procurved, tipped towards the other; fertilization duct winds twice around base of bursa; spermathecae are touching or nearly touching; ventral orientation of connecting tube as it enters spermatheca with a straight entry pattern; spermatheca is tear drop-shaped with a longitudinal orientation and lies dorsally of bursa; diverticle is a curved, thickened tube, much larger than spermatheca, widely set from the other; anterior atrial edge is smooth; epigynal bridge is thin and wide; bursa opening is partially visible when viewed straight on (Figs. 32, 47).

Distribution.—CANADA: *Alberta, British Columbia, Saskatchewan*; USA: *Oregon, Washington* (Fig. 61C).

Material examined.—CANADA: *Alberta*, Little Smokey River, 10 km S. of Guy, 4 August 1965, 3♀, 2♂, J. Ivie & W. Ivie, AMNH. *British Columbia*, Wellington, Vancouver Island, 49°N, 123°W, 5 September 1949, 1♀, R. Guppy, AMNH. *British Columbia,* Wellington, Vancouver Island,

49°N, 123°W, 20 September 1949, 1 ♀, R. Guppy, AMNH. *British Columbia*, Wellington, Vancouver Island, 49°N, 123°W, 20–24 August 1950, 1♀, R. Guppy, AMNH. *British Columbia*, Wellington, Vancouver Island, 49°N, 123°W, September 1950 (no day listed), 2♀, R. Guppy, AMNH. *British Columbia*, East side Saanich Inlet, 48°N, 123°W, collection date is probably 14 September 1935, 4♀, no collector listed, AMNH. *British Columbia*, East side Saanich Inlet, 48°N, 123°W, 14 September 1935, 4♀, no collector listed, AMNH. *British Columbia*, East side Saanich Inlet, 48°N, 123°W, 14 September 1935, 4♀, R. Chamberlin & W. Ivie, AMNH. *British Columbia*, West side Saanich Inlet and Sidney, Vancouver Island, 48°N, 123°W, 16 September 1935, 5♀, R. Chamberlin & W. Ivie, AMNH. *British Columbia*, Vancouver Island, Department of National Defense Rocky Point, 48.32°N, 123.55°W, 4 October 1995, malaise trap, garry oak, open meadow, 1♂, N. Winchester, DMNS ZA.31407. *Saskatchewan*, Anglin Lake, 53.73°N, 105.93°W, 29 July–19 August 1998, grassy hillside, pitfall traps, 3♂, D.J. Buckle, DMNS ZA.31366. *Saskatchewan*, Borden Bridge, 52.37°N, 107.15°W, 26 August 1985, 1♂, D.J. Buckle, DMNS ZA.31365. *Saskatchewan*, 14 km SSW Dundurn, 51.68°N, 106.58°W, 22 July 1972, slough margin, 1♂, D.J. Buckle, DMNS ZA. 14495. *Saskatchewan*, Hodgeville, 719 m, 50.11°N, 106.97°W, 22 July–10 August 1996, pitfall traps, rail line, 1♂, K. Pivnick, DMNS ZA.31400. *Saskatchewan*, Hodgeville, 50.12°N, 106.98°W, 10–27 August 1996, pitfall traps, 1♂, K. Pivnick, DMNS ZA.31405. *Saskatchewan*, Hodgeville, 719 m, 50.11°N, 106.97°W, 1–21 August 1995, rail line, pitfall traps, 1♂, K. Pivnick, DMNS ZA.31399. *Saskatchewan*, Lady Lake, 51.05°N, 102.63°W, summer 1963, 1♂, M. Buckle, DMNS ZA.31395. *Saskatchewan*, 8 km east of Marcelin, 52.82°N, 109.70°W, 11 August 1986, 1♂, D.J. Buckle, DMNS ZA.31411. *Saskatchewan*, North Battleford, 52.77°N, 106.67°W, 7–26 August 1996, pitfall traps, 3♂, K. Pivnick, DMNS ZA.31402. *Saskatchewan*, Radville, 625 m, 49.46°N, 104.29°W, 20 July 1967, 1♂, D.J. Buckle, DMNS ZA. 31398. *Saskatchewan*, Saskatoon, 52.13°N, 106.67°W, 13 July 1980, in web in tall grass, 1♂, D.J. Buckle, DMNS ZA.31403. *Saskatchewan*, Saskatoon, 52.13°N, 106.67°W, 13 August 1982, 2♂, 3♀, D. J. Buckle, DMNS ZA.31409. *Saskatchewan*, 8 km NE of Saskatoon, 505 m, 52.23°N, 106.52°W, 10–17 August 1967, 5♂, E. Corin, ZA.31397. *Saskatchewan*, St. Denis, Champetre, 560 m, 52.16°N, 106.10°W, 8–23 August 1996, native grass & trees, pitfall traps, 3♂, K. Pivnick, DMNS ZA.31401. *Saskatchewan*, St. Denis, 52.16°N, 106.10°W, 560 m, 20 July–7 August 1955, cultivated trees, pitfall traps, K. Pivnick, 1♂, DMNS ZA.31408. USA: *Oregon*, Benton County, Corvallis, 44°N, 123°W, 1 November 1950, 1♀, V. Roth, AMNH. *Oregon*, Benton County, 19 km north of Corvallis, 44°N, 123°W, 25 September 1947, 1 ♀, V. Roth, AMNH. *Oregon,* Benton County, 44°N, 123°W, 28 September 1952, 1 ♀, G. Konnersman, AMNH. *Oregon*, Jackson County, 42°N, 122°W, 1935, 1♀, F. Lawrence, AMNH. *Oregon*, Lane County, Eugene, 44°N, 123°W, 13 September 1941, Spencer Butte, 1♀, B. Malkin, AMNH. *Washington*, Grays Harbor County, Ford Prairie, 46.87°N, 123.29°W, 20 June 2007, 15–18 m, from ground web on prairie; reared–2 molts, 1♂, L. Ramseyer, UWBM.2. *Washington*, Island County, Oak Harbor, 18 September 1935, 1♀, C.H. Harrison, UWBM.12.

Agelenopsis aleenae (Chamberlin & Ivie 1935)

Agelenopsis aleenae Chamberlin & Ivie 1935: 33, pl. 14, f. 111.

Figs. 1, 2, 34, 49, 61D

Type specimens.—Male holotype examined: *New Mexico*: Valencia County, Suwanee (Correo) 35°N, 107.08°W, 6 September 1933, found under a rock on a dry hillside, Aleen Ivie, AMNH.

Etymology.—Named for Aleen Ivie, wife of arachnologist Wilton Ivie, who collected the specimen.

Diagnosis.—The male *A. aleenae* is distinguished by its tight coiling embolus, diameter of coiling not substantially wider than cymbium width, and spatulate embolic tip (Figs. 1, 2). The conducting tube in the female is unique in its flared and thickened shape as it emerges high in the bursa's structure and descends to its base where it enters the spermatheca (Fig. 49, "ct").

Description.—General description as for the genus. Male (n = 10): overall length 8.85–13.80 mm; carapace width at its widest point 3.13–4.33 mm; carapace width at its narrowest point 1.71–2.28 mm. Shape of RTA is nipple–like; shape of median apophysis is thick, but somewhat pointed; conductor size is large with generally pointed shape; coiling of embolus is tight with an embolic tip termination angle of 540°; embolus subtriangular segment is absent; embolic tip orientation is not recurved; embolic tip is twisted and spatulate; anneli on subtegulum are present (Figs. 1, 2). Female (n = 24): overall length 8.90–15.95 mm; carapace width at its widest point 3.03–4.61 mm; carapace width at its narrowest point 1.59–2.64 mm. Bursa is longer than wide, tip folded strongly ventrally, narrowly separated from or touching other bursa; spermatheca is elongated and kidney shaped; two turns of the fertilization ducts; spermathecae are touching, oriented longitudinally, and sit centrally between bursae; conducting tube emerges and descends steeply from upper portion of bursa, then splits, thickens (obscuring view of almost entire surface of spermatheca), and converges entering spermatheca vertically, but at posterior (bottom) edge; very long conducting tube on ventral side; diverticle is bulbous and same size as spermatheca, and does not touch the other; anterior atrial edge is monolobed; epigynal bridge is thin and wide; bursa opening is mostly visible (Fig. 34, "bo").

Distribution.—USA: *Colorado, Kansas, New Mexico, Texas* (Fig. 61D).

Material examined.—USA: *Colorado*, Cheyenne County, Kit Carson, 38.76°N, 102.79°W, 19 September 1999, grassland, 1♀, 1♂, L. & M. Siderhurst, DMNS ZA.11437. *Colorado*, Las Animas County, Piñyon Canyon Maneuver Site, off Rd. 4 A, 37.42°N, 103.85°W, 13 September 2006, 1539 m, 1♀ , P.E. Cushing, DMNS ZA.11491. *Colorado*, Las Animas County, Piñyon Canyon Maneuver Site, canyon feeding into Toyler Arroyo near Biernack Barracks, 37.41°N, 103.88°W, 12 September 2006, 1524 m, 21:00–22:30 hr, headlamping, 1♂, J. Slowik, DMNS ZA.14493. *Colorado,* Las Animas County, Piñyon Canyon Maneuver Site, windmill of Rd. 3A, 13 September 2006, 37.42°N, 103.94°W, 1515 m, 16:00–16:30 hr, lookdown, J. Slowik, DMNS ZA.14494. *Colorado,* Las Animas County, Piñyon Canyon Maneuver Site, windmill along Road 2, 37.39°N, 104.03°W, 13 September 2006, 1546 m, short grass prairie, moist site, 1♀, J. Demboski, DMNS ZA.14495. *Colorado*, Las Animas County, Piñyon Canyon

Maneuver Site, off Rd. 3A, 37.42°N, 103.94°W, 13 September 2006, 1515 m, 16:20–16:40 hr, 1♀, P.E. Cushing, DMNS ZA.14496. *Colorado,* Las Animas County, off Rd. 4, 37.43°N, 103.94°W, 13 September 2006, 1544 m, 1♀, J. Demboski, DMNS ZA.14497. *Colorado,* Otero County, Comanche National Grasslands, 21 km S of La Junta, 5 km W of CR 802, David Canyon Rd., 37.79°N, 103.52°W, 19 September 2009, 1360 m, lookdown, 12:10–16:30 hr, open short grass, 4♀, M. Francis & J. Whitman–Zai, DMNS ZA.22371.

Colorado, Prowers County, Lamar, Road DD, 38.09°N, 102.62°W, 15 September 2003, trap 7, 8, pitfall, 1104 m, 1♂, H. Miller, DMNS ZA.21314. *Colorado*, Prowers County, Lamar, Rd. DD, 38.09°N, 103.61°W, 22 September 2005, 1104 m, trap 13, 14, fallow alfalfa field, 1♂, L. Kerzicnik, DMNS ZA.21319. *Colorado*, Prowers County, Lamar, Rd. DD, 38.09°N, 103.62°W, 22 September 2005, 1104 m, trap 21, 22, fallow sorghum field, 1♂, L. Kerzicnik, DMNS ZA.21487. *Colorado*, Prowers County, Lamar, 38.09°N, 102.62°W, 3 October 2006, Rd. DD, 1104 m, trap 25, 26, pitfall, 1♀, L. Kerzicnik, DMNS ZA.21486. *Colorado*, Prowers County, S of Lamar, off Trail Rd. by deserted house off CR 2, N 37.97°N, 102.72°W, 18 September 2009, 4♀, 1197 m, lookdown, 13:20–14:22 hr, M. Francis & J. Whitman-Zai, DMNS ZA.22370.

Kansas, Meade County, Meade State Park, 37.17°N, 100.45°W, 15 August 2004, 1♂, N. Ayoub, CAS. *New Mexico*, Colfax County, Farley, 2 August 1991, under boards & sheet metal at abandoned farm, 1♀, 1♂, H.S. Fitch, HJG.649. *New Mexico*, Colfax County, 5 km N of Farley off County Road 193, 37.23°N, 104.49°W, 5 August 2009, look down in weeds by culvert, 1♀, M. Francis & J. Whitman–Zai, DMNS ZA.22599. *New Mexico,* Colfax County, 7 km N of Farley, County Road 193 before mile marker 7, 37.40°N, 104.48°W, 5 August 2009, 1♀, 1846 m, look down, 15:00–15:30 hr in weeds near culvert, M. Francis & J. Whitman-Zai, DMNS ZA.22173. *New Mexico,* Colfax County, 7 km N of Farley, 6–7 August 2009, CR 193 before mile marker 7, 37.22°N, 104.48°W, 1846 m, pitfall in weeds near culvert, 2♀, M. Francis & J. Whitman-Zai, DMNS ZA.22174. *New Mexico*, Union County, off Morrow Rd. just W of Capulin National Monument, 36.78°N, 103.99°W, 7 August 2009, 2218 m, look down, 14:30–14:40 hr, 1♀, M. Francis & J. Whitman-Zai, DMNS ZA.22598. *Texas*, Howard County, Big Spring, random salt cedar, 17 September 2006, 1♀, E.M. Knutson, NMSU.992. *Texas*, Llano County, 30°N, 98°W, 2♀, 1 October 1950, 1♂, W.J. Gertsch, AMNH. *Texas*, Reeves County, 16 km S of Balmorhea, 30.85°N, 103.80°W, 19 May 2003, 1♂, N. Ayoub et al., CAS.

Agelenopsis aperta (Gertsch 1934)
Ageleua aperta Gertsch 1934: 25, f.10.
Figs. 23, 24, 41, 56, 57, 61D

Type specimens.—Male holotype and female allotype examined: *Colorado*, Boulder County, Valmont Buttes, east of Boulder, 27 July 1908, 40.07°N, 105.13°W, F.E. Lutz, AMNH Paratypes examined: *Colorado*, Boulder County, Valmont Buttes east of Boulder, 27 July 1908, 40.07°N, 105.13°W, F.E. Lutz, AMNH; *Califoruia*: Los Angeles County, Los Angeles, November, December 1931, Grant, AMNH; *Texas*, Travis County, Austin, Cockrell, September 1901, Petrunkevitch, AMNH; *Utah,* Salt Lake County, Salt Lake City July, September 1931, AMNH; *Utah*, Washington County, Zion National Park, 4 July 1931, Gertsch, AMNH.

Etymology.—None provided but "apertum" in Latin means "opening" and may refer to the distinctive atrium of the female.

Diagnosis.—The male *A. aperta* is distinguished from other species in the genus by its loosely coiling embolus, making more than one full circle, a procurved and twisting tip, and a pointed conductor tip (Figs. 23, 24). The *A. aperta* female has a unique diverticle and conducting tube that diverges and converges before entering the spermatheca, remaining thin and distinctive Figs. 56, 57).

Description.—General description as for the genus. Male (u = 29): overall length 9.40–15.14 mm; carapace width at its widest point 3.19–5.14 mm; carapace width at its narrowest point 1.74–3.81 mm. Shape of RTA is nipple–like; shape of median apophysis is thick and somewhat pointed; conductor size is large with generally pointed shape; coiling of embolus is loose with an embolic tip termination angle of 540°; embolus subtriangular segment is absent; embolic tip orientation is not recurved; embolic tip is twisted; anneli on subtegulum are present (Figs. 23, 24). Female (u = 27): overall length 11.03–15.92 mm; carapace width at its widest point 3.57–5.44 mm; carapace width at its narrowest point 1.92–3.34 mm. Bursa longer than wide, oriented vertically, tips ventrally, narrowly separated from or touching other bursa; spermatheca is tear-shaped, set centrally with respect to bursa, and oriented longitudinally; spermathecae touching; two turns of fertilization ducts are visible; conducting tubes diverge and then converge before entering spermatheca centrally on dorsal side from a vertical position; diverticle is a curved, thickened tube much larger than spermatheca and slightly separated from other; anterior atrial edge is slightly bilobed; epigynal bridge is thin and wide; bursa opening is mostly visible (Figs 41, 56, 57).

Distribution.—USA: *Arizoua*, *Califoruia*, *Colorado*, *Nebraska*, *New Mexico*, *Nevada*, *Oklahoma*, *Utah*, *Texas* (Fig. 61D).

Material examined.—USA: *Arizoua*, Cochise County, SWRS, Chiricahua Mtns., 31°N, 109°W, 26 August 1955, 1♀, 1♂, W.J. Gertsch, AMNH. *Arizoua*, Cochise County, SW Research Station, 8 km west of Portal, 25 July 1956, 1♀, M.J. Westfall, AMNH. *Arizoua*, Cochise County, 8 km west of Portal, SW Research Station, 7 August 1956, 1♂, M.J. Westfall, AMNH. *Arizoua*, Cochise County, SWRS, Chiricahua Mtns., 31°N, 109°W, 10 August 1956, 1♂, no collector listed, AMNH. *Arizoua*, Cochise County, Portal SW Research Station, 27 August 1958, on building, 1♀, H.V. Weems, Jr., AMNH. *Arizoua*, Cochise County, 8 km W of Portal, SW Research Station, 10 September 1958, 1♂, H.V. Weems, Jr., AMNH. *Arizoua*, Cochise County, Portal, Chiricahua Mtns, 31°N, 109°W, 18 June 1955, 1♂, no collector listed, AMNH. *Arizoua*, Cochise County, Portal, Chiricahua Mtns., 31°N, 109°W, 18 June 1955, 1♂, M. Statham, AMNH. *Arizoua*, Cochise County, 31°N, 109°W, no collection date listed, 1♀, no collector listed, AMNH. *Arizoua*, Cochise County, Portal 1, 1455 m, 31.91°N, 109.14°W, 1 July 1978, 1♂, B. & C. Durden, DMNS ZA.137. *Arizoua*, Cochise County, Herb Martyr Dam S. of Portal, 31.87°N, 109.23°W, 9 August 1972, 1♂, B. Vogel, DMNS ZA.11808. *Arizoua*, Maricopa County, 2 km N of

New River September 1966, 1♀, 1♂, no collector listed, MCZ.39996.

Arizona, Pinal County, San Manuel, 32 km S of Redington, August 1977, 1♂, W.J. Gertsch, AMNH. *California*, Los Angeles County, 10 August 1932, La Crescenta, 1♀, W. Ivie, AMNH. *California*, Los Angeles County, San Fernando, 34.28°N, 118.47°W, 14 September 1964, 2♀, W. Ivie, AMNH. *California*, Los Angeles County, Sepulveda Canyon, 34°N, 118°W, 17 September 1941, 1♀, W. Ivie, AMNH. *California*, Orange County, Banning Canyon, 33°N, 116°W, 18 May 1951, 1♀, 1♂, E. Schlinger, AMNH. *California*, Tulare County, Three Rivers, 250 m, 21 June 1985, 2♀, 3♂, H. & L. Levi, MCZ.39997. *Colorado*, Adams County, Aurora, 63485 S Yellowstone Court, 80016, 39.60°N, 104.72°W, 6–9 September 2000, 1♀, G. Schmidt, DMNS ZA.13345. *Colorado*, Arapahoe County, Aurora, 18939 E. Warren Circle, #102, 39.83°N, 104.76°W, 22 August 2003, 1♂, B. Nelson, DMNS ZA.6284. *Colorado*, Arapahoe County, Greenwood Village, 100 Blue Heron Court, 39.62°N, 104.93°W, 3 October 2000, 1♀, A. Gilden, DMNS ZA.4964. *Colorado*, Arapahoe County, Old Littleton, 39.61°N, 105.02°W, 1643 m, 12 June 2001, 1♂, T. Adair, DMNS ZA.11442. *Colorado*, Boulder County, 40.02°N, 105.31°W, 13 July 1962, 1♀, B. Vogel, DMNS ZA.1913. *Colorado*, Boulder County, 18 July 1962, 2♂, B. Vogel, CU.6. *Colorado*, Boulder County, South of County Rd. 67, 39.91°N, 105.27°W, 5 July 1999, 1♂, 10:00–14:00 hr, 4 hour cumulative, look down; 2 hr cumulative look up, L. & M. Siderhurst, DMNS ZA.11382. *Colorado*, Douglas County, Roxborough State Park near visitor center, 39.42°N, 105.05°W, 18 September 1999, 1♀, 11:00–12:00 hr, R. Burleigh, DMNS ZA.13364. *Colorado*, Douglas County, Sedalia, 2691 W. Wolfensberger Rd., 1775 m, 39.44°N, 104.96°W, 15–18 August 1998, 1♂, G. Dennison, DMNS ZA.4972. *Colorado*, El Paso County, 2 km W of Monument off Mt. Herman Rd. 320, 39.08°N, 104.89°W, 07 September 2001, 1♀, 2101 m, B. Morrison, DMNS ZA.4786. *Colorado*, Elbert County, Elizabeth, 627 Panorama Dr., 39.36°N, 104.60°W, 31 July–19 August 2002, 1♂, casual, C.J. Bishop, DMNS ZA.9040. *Colorado*, Jefferson County, Butterfly Pavilion and Insect Center, Westminster, 39.88°N, 105.05°W, 9 July 2005, 1♂, 9 July 2005, 1♂, P.E. Cushing & F. Haas, DMNS ZA.11209. *Colorado*, Jefferson County, Morrison, 5591 Willow Wood, 39.63°N, 105.27°W, 2 July 2002, 1♂, R. L. Harwood, DMNS ZA.4977. *Colorado*, Montezuma County, Mesa Verde NP, around research center, 37.15°N, 108.51°W, 31 July 2001, 1♀, casual collecting, A.R. Nabors, DMNS ZA.11974. *Nebraska*, Buffalo County, Amherst, October 1971, 1♀, L. Alexander, MCZ.40000. *Nevada*, Clark County, Las Vegas, 36°N, 115°W, 27 July 1944, 1♀, D. Zinn, AMNH. *Nevada*, Washoe County, Reno, U of Nevada College Quad, 39°N, 119°W, July 1940, 1♀, no collector listed, AMNH. *New Mexico*, Doña Ana County, November 1980, 1♀, 1♂, Las Cruces, no collector listed, USNM.A2. *New Mexico*, Hidalgo County, Gray Ranch Survey, Upshaw Camp, draw to S., 8 August 1991, 1♂, D. Richman, NMSU.A3. *New Mexico*, Hidalgo County, 8 August 1991, 1♂, Upshaw Camp; draw to south, D. Richman et al., NMSU.B2. *New Mexico*, Otero County, High Rolls Mtn. Park, 4 April 1966, 1♂, L. Pinter, MCZ.39998. *Oklahoma*, Comanche County, Fort Sill, Lake Elmer Thomas Recreation Area (LETRA), 34.72°N, 98.53°W, 442 m, 20 September 2003, 1♀, lookdown 11:40–12:40 hr, P.E. Cushing, DMNS ZA.7056. *Texas*, Bandera County, 29°N, 99°W, 1 August 1940, 1♀, D. & S. Mulaik, AMNH. *Texas*, El Paso County, Clint, 31°N, 106°W, 9 June 1939, 1♀, I.R. Davis, AMNH. *Texas*, Fort Bend County, Needville, 17 June 1978, 1♂, in web at base of house, D.A. Dean, TXAM.535. *Texas*, Travis County, Austin, 30.39°N, 97.73°W, 25 August 1968, 2♀, B. Vogel, DMNS ZA.154. *Utah*, Salt Lake, 1936 (only year provided on label), 1♀, M.J. Westfall, AMNH.

Agelenopsis emertoni (Chamberlin & Ivie 1935)

Agelenopsis emertoni Chamberlin & Ivie 1935: 33, pl. 14, f.110.

Figs. 25, 26, 42, 58, 61C

Type specimens.—Male holotype, female allotype examined, *Texas*, Bell County, Belton, 31.07°N, 97.28°W, 1 September 1933, W. Ivie, AMNH.

Etymology.—Named for arachnologist James H. Emerton.

Diagnosis.—The male *A. emertoni* is distinguished from other species in the genus by its loosely coiling embolus, making more than one full circle, a procurved and twisting tip, and a claw-like conductor tip (Figs. 25, 26). The female is distinctive for having a conducting tube that descends straight from bursa but angles sharply ectally to enter a spermatheca with a flanged tip (Fig. 58).

Description.—General description as for the genus. Male (n = 28): overall length 6.00–12.57 mm; carapace width at its widest point 2.25–4.34 mm; carapace width at its narrowest point 1.12–2.23 mm. Shape of RTA is nipple-like; shape of median apophysis is thick and somewhat pointed; conductor size is large with a claw-like shape; coiling of embolus is loose with an embolic tip termination angle of 540°; embolus subtriangular segment is present; embolic tip orientation is not recurved; embolic tip is twisted; anneli on subtegulum are present (Figs. 25, 26). Female (n = 27): overall length 7.20–15.17 mm; carapace at its widest point 2.20–4.82 mm; carapace at its narrowest point 1.15–2.81 mm. Bursa is generally rigid and inflated, oriented ventrally; bursae narrowly spaced or touch distally but more widely set at base; spermatheca is kidney-bean shaped but narrows and is flanged dorsally, is separated from the other, sits dorsally of bursae, and oriented generally longitudinally; two turns of fertilization ducts visible; conducting tube descends from bursa to spermatheca straightforwardly and then angles ectally toward outer sides on anterior edges where it enters the spermatheca; diverticle is a eurved, thickened tube, larger than the spermatheca, and touches other diverticle; anterior atrial edge is slightly bilobed; epigynal bridge is thin and wide; bursa opening is mostly visible (Figs. 42, 58).

Distribution.—USA: *Arkansas*, *Colorado*, *Florida*, *Georgia*, *Louisiana*, *Massachusetts*, *Mississippi*, *Missouri*, *New Jersey*, *New York*, *North Carolina*, *Oklahoma*, *Pennsylvania*, *Tennessee*, *Texas*, *Virginia* (Fig. 61C).

Material examined.—USA: *Arkansas*, Lawrence County, Imboden, 36.2°N, 91.17°W, 1935 (only collection year listed), 3♀, 1♂, A. C. Marshall, AMNH. *Colorado*, Douglas County, Castlewood Canyon State Park, 39.35°N, 104.76°W, 14 August 1999, 1♀, 11:00–12:30 hr, J. Dickinson, DMNS ZA.4774. *Colorado*, Jefferson County, Littleton, Chatfield SP, Hwy 121 & C470, 39.52°N, 105.08°W, 7 October 2004, 1♀, 1580 m, under bark of cottonwoods & willows, B. Morrison,

DMNS ZA.8165. *Colorado*, Montezuma County, Mesa Verde NP, Chapin Mesa, 39.18°N, 108.15°W, 5 June–30 September 2006, 1♂, 2135 m, inside dwelling, D.L. Ely, DMNS ZA.12950. *Florida*, Alachua County, Gainesville, DPI (Division of Plant Industry), 3 November 1981, 1♂, G.B. Edwards, FSCA. *Florida*, Alachua County, Gainesville, DPI, 19 December 1981, 3♂, G.B. Edwards, FSCA. *Georgia*, Ben Hill County, Fitzgerald, in pecan grove, 12 September 1950, 1♂, M. Hopkins, FSCA. *Louisiana*, Baton Rouge Parish, 30°N, 91°W, July 1955, 1♀, 1♂, R.V. Chamberlin & W. Ivie, AMNH. *Massachusetts*, Barnstable County, Quisset Salt Pond, 8 October 1988, 1♀, R. L. Edwards, USNM. *Mississippi*, Tishomingo County, Luka, 34°N, 88°W, 5 September 1941, 1♂, C. Goodnight, AMNH. *Missouri*, Vernon County, Nevada, 8 October 1962, 1♀, 1♂, on store front, J.W. McReynolds, MCZ.39986. *New Jersey*, Bergen County, Ramsey, 10 October 1933, 2♂, 41°N, 74°W, W.J. Gertsch, AMNH. *New Jersey* Monmouth County, Red Bank, September 1958, 1♀, R. Willey, MCZ.39988. *New York*, Suffolk County, Greenport, Long Island, 41°N, 72°W, September 1957, 1♂, R. Latham, AMNH. *New York*, Suffolk County, 22 September 1922, 2♂, Riverhead, 40°N, 72°W, M.I. King, AMNH. *North Carolina*, Durham County, Duke Forest, summer 1938, 1♀, A.M. Chickering, MCZ.39995. *Oklahoma*, Comanche County, Fort Sill, E. Cache Creek, 34.64°N, 98.36°W, 3 October 2004, 1♀, 326 m, 12:00–13:00 hr, in woods above stream, look down, P.E. Cushing, DMNS ZA.7339. *Oklahoma*, Payne County, Stillwater, 36°N, 96°W, 1936 (only collection year listed), 1♀, 1♂, C. Smith, AMNH. *Pennsylvania*, Bucks County, Neshaminy Creek, 0.6 km east of Jamison, 40.16°N, 75.03°W, September 1952, 2♀, 2♂, no collector listed but probably W. Ivie, AMNH. *Pennsylvania*, Bucks County, Neshaminy Creek, northeast of Jamison, 40.16°N, 75.30°W, 16 September 1962, 3♀, no collector listed but probably W. Ivie, AMNH. *Pennsylvania*, Bucks County, NE of Jamison, Horseshoe Bend, Neshaminy Creek, 40.27°N, 75.05°W, August 1956, 4♀, 4♂, W. Ivie, AMNH. *Pennsylvania*, Columbia County, Orangeville, 41°N, 76°W, August 1931, 1 ♂, Hughes, AMNH. *Tennessee*, Jackson County, Cherry Cove, Bailey, 19 October 1948, 1♀, Jones & Archer, AMNH. *Texas*, Bastrop County, Little Sandy Creek, 16 km NW Bastrop, 28 October 1971, 1♂, B. Vogel, DMNS ZA.150. *Texas*, Belton County, 31°N, 97°W, 01 September 1933, 1♀, 1♂, W. Ivie, AMNH. *Texas*, Dallas County, White Rock, 10 September 1939, 1♂, V. Roth, MCZ.39994. *Texas*, Grayson County, 10 km N of Denison, 33.52°N, 96.34°W, 20 October, 1963, 3♀, K.W. Haller, AMNH. *Texas*, Nusces County, 6 km NW Port Aransas, 8 October 1972, 1♀, moist salt beach, B. Vogel, DMNS ZA.146. *Virginia*, Albemarle County, 27 August 1948, 1♂, H.K. Wallace, FSCA.

Agelenopsis kastoni (Chamberlin & Ivie 1941)
Agelenopsis kastoni Chamberlin & Ivie 1941: 591, pl. 2, fig. 18, 35.
Figs. 19, 20, 39, 54, 61A

Type specimens.—Male holotype, female allotype examined. *Connecticut*, Middlesex County, Haddam, 41.47°N, 72.52°W, 27 May 1935, B. J. Kaston, AMNH.

Etymology.—Named for arachnologist Benjamin J. Kaston who collected the holotype.

Diagnosis.—The male of this species is distinguishable from others in the genus by its loosely coiling, recurved embolus, making more than one full circle and ending in a smooth, tapering tip (Figs. 19, 20). The female *A. kastoni* is distinctive in having a particularly dark, sclerotized fertilization duct and spermatheca that is thickened and flanged out on dorsal side (Fig. 54).

Description.—General description as for the genus. Male (n = 22): overall length 6.20–8.80 mm; carapace width at its widest point 2.00–2.95 mm; carapace width at its narrowest point 1.22–1.58 mm. Shape of RTA is nipple–like; shape of median apophysis is thick and somewhat pointed; conductor size is large with generally pointed shape; coiling of embolus is loose with an embolic tip termination angle of 540°; embolus subtriangular segment is absent; embolic tip orientation is recurved; embolic tip is slender and tapering; anneli on subtegulum are present (Figs. 19, 20). Female (n = 52): overall length 4.00–10.29 mm; carapace width at its widest point 1.49–2.90 mm; carapace width at its narrowest point 0.82–1.71 mm. Bursa angles toward other, touching distally but set wide apart at base, vertically oriented when viewed from above; spermatheca is kidney shaped, oriented longitudinally, nestled centrally in bursae; spermathecae touching; two turns of fertilization ducts visible; diverticle is bulbous and same size as spermatheca; slightly separated from other; conducting tube pattern complex with convoluted loops; anterior atrial edge is bilobed; epigynal bridge is thin and wide; bursa opening is mostly visible (Figs. 39, 54).

Distribution.—USA: *Arkansas*, *Connecticut*, *Florida*, *Georgia*, *New Jersey*, *New Mexico*, *Massachusetts*, *Maryland*, *Missouri*, *North Carolina*, *Tennessee*, *West Virginia* (Fig. 61A).

Material examined.—USA: *Arkansas*, Logan County, Mt. Magazine, Mossback Ridge, north slope, 23 June 1990, pitfall, 1♀, B. Leary, AMNH. *Arkansas*, Washington County, Cove Creek Valley, 24 km west of Prairie Grove, Boston Mtns. March–April 1956, 12♀, 5♂, 305 m, M. Hite, MCZ.39968. *Arkansas,* Washington County, Cove Creek, 12 May 1963, 1♀, O. & M. Hite, Whitcomb, & Frizzell, AMNH. *Connecticut*, Middlesex County, Haddam, 27 May 1935, 1♀, 1♂, B. J. Kaston. *Connecticut*, New London County, Waterford, 30 June 1935, 1♂, B.J. Kaston, AMNH. *Florida*, Liberty County, Torreya State Park, 2 April 1963, 2♂, W. Shear, AMNH. *Florida*, Liberty County, Torreya State Park, 21 km north of Bristol, 18 December 1967, 1♂, W. Ivie, AMNH. *Georgia*, Clayton County, NW Clayton, 34.88°N, 83.47°W, 28 April 1943, 12♀, W. Ivie, AMNH. *Georgia*, Habersham County, Clarkesville, 34.60°N, 83.52°W, 27 April 1943, 4♀, W. Ivie, AMNH. *Georgia*, Habersham County, Tallulah Falls, 34.75°N, 83.42°W, 27 April 1943, 4♀, W. Ivie, AMNH (2 sets of vials with same collection information and 4♀s each). *Georgia*, Habersham and Stephens Counties, Clarkesville to Toccoa, 34.58°N, 83.42°W, 28 April 1943, 4♀, W. Ivie, AMNH. *Georgia*, Hall County, 8 km NE of Gainesville, 34.33°N, 83.77°W, 26 April 1943, 2♀, W. Ivie, AMNH. *Georgia*, Rabun County, NW Clayton, 34.88°N, 83.47°W, 28 April 1943, 4♀, 4♂, W. Ivie, AMNH. *New Jersey*, Burlington County, Lebanon State Forest, 39.55°N, 74.37°W, 10 May 1964, 1♀, J. & W. Ivie, AMNH. *Maryland*, Prince Georges County, Patuxent Game Refuge, Bowie, 39°N, 76°W, May 1941, 1♂, L.W. Saylor, AMNH. *Maryland*, Prince Georges

County, 27 May–4 June 1978, 1♂, J.F. Reinert, USNM. *Massachusetts*, Barnstable County, Hatchville, FCWMA, 28 July 1989, pitfall, deciduous woods, 1♀, R.L. Edwards, USNM. *Massachusetts*, Plymouth County, Wareham, 17 June 1960, 1♂, B.J. Kaston, USNM. *North Carolina*, Durham County, Kerley Rd., about 213 m west of 751, Duke Forest (stand 25 years old), 23 April 1964, pitfalls, pine with young hardwood, 1♂, J.W. Berry, USNM. *Tennessee*, Anderson County, Norris, 1 May 1975, rock outcrop, found on sheet web, 1♂, A. Kronk, USNM. *West Virginia*, Berkeley County, Sleepy Creek Hunt and Fish Area, Third Hill Mtn., 16–23 May 1986, 1♂, unbaited pitfall trap, P5 New S.W., P.J. Martinat, USNM. *West Virginia*, Berkeley County, Sleepy Creek Hunt & Fish Area, Third Hill Mountain, 23–30 May 1986, oak-pine forest, unbaited pitfall trap, 1♂, P.J. Martinat, USNM. *West Virginia*, Berkeley County, 1986, Sleepy Creek Hunt & Fish Area, Third Hill Mountain, 20–27 June 1986, unbaited pitfall trap, 5 New NW, 1♀, J.P. Martinat, USNM.

Agelenopsis longistyla (Banks 1901)
Ageleua longistylus Banks 1901: 576.
Figs. 15, 16, 40, 55, 61B

Type specimens.—Male holotype not examined. *New Mexico*, Lincoln County, White Mountains, first Ruidoso camp, 10 August, 1901, C.H.T. Townsend.

Etymology.—Named for the male's distinctive, long, hooked embolic tip.

Diagnosis.—The male *A. longistyla* is distinguishable from other species in this genus by its loosely coiling embolus, with pronounced procurved, hooked tip (Figs. 15, 16). The female, like A. *aleenae*, is distinguishable from most other species in this genus by its very wide set bursae but is the only species with a curved tubular diverticle that is thin (Fig. 55).

Description.—General deseription as for the genus. Male (n = 17): overall length 6.2–8.3 mm; carapace width at its widest point 1.81–2.59 mm; carapace width at its narrowest point 1.01–1.51 mm. Shape of RTA is nipple-like; shape of median apophysis is thick and somewhat pointed; conduetor size is large with generally pointed shape; coiling of embolus is loose with an embolie tip termination angle of 720°; embolus subtriangular segment is absent; embolic tip orientation is not recurved; embolic tip is hooked with notch; anneli on subtegulum are present (Figs. 15, 16). Female (n = 58): overall length 2.6–3.88 mm; carapace width at its widest point 1.83–2.8 mm; carapace width at its narrowest point 1.0–1.47 mm. Bursa is longer than wide, oriented ventrally, and widely spaced from other; spermatheca is tear-shaped, widely set apart from the other, longitudinally oriented, and nestled between bursae; two turns of fertilization ducts are visible; conducting tube enters spermatheca at the very top (anterior edge); diverticle is curved, thin tube about the same size as spermatheca and widely spaced from other; anterior atrial edge distinctly bilobed; epigynal bridge thin and wide; bursa opening partially visible (Figs. 40, 55).

Distribution.—USA: *Arizona*, *Colorado*, *New Mexico*, *Texas* (Fig. 61B).

Material examined.—USA: *Arizona*, Navajo County, Overgaard Camp, 10 km north of Heber, 34°N, 110°W, 17 September 1950, 2♀, W.J. Gertsch, AMNH. *Colorado*, Alamosa County, San Luis Lakes State Park, 37.67°N, 105.72°W, 11 September 1999, 11:00–12:30 hr, 1♀, P.E. Cushing, DMNS ZA.13352. *Colorado*, Conejos County, 18 Rd Antonito, 37.08°N, 106.01°W, 9 September 2001, under rock, 1♀, N. Betzen, DMNS ZA.13471. *Colorado*, Douglas County, Castle Rock, Gateway Mesa Park, 39.39°N, 104.80°W, 24 August 2005, 1951 m, look down in grass, 12:59 hr, foothills, 1♂, B. Morrison, DMNS ZA.12048. *Colorado*, Saguaehe County, Friendly Gulch, 38.10°N, 106.18°W, 9–18 September 1999, 4♂, P.M. Pineda, DMNS ZA.9036. *Colorado*, Saguaehe County, Friendly Gulch, 38.10°N, 106.18°W, 9–18 August 1999, 4♂, pitfall trap, P.M. Pineda, DMNS ZA.9037. *Colorado*, Weld County, C.P.E.R. Pawnee National Grassland, Pasture 23 W, 11 August 1994, 1♂, R.D. Weeks, Jr., NMSU.190. *New Mexico*, Grant County, 1♂, Burro Mtns., piñon juniper/nolina, 1 September 1973, M.H. Muma, FSCA.9. *New Mexico*, Grant County, Emory Pass Summit, Nimbres Mtns., 32.83°N, 107.75°W, 6 September 1941, 4♀, W. Ivie, AMNH. *New Mexico*, Grant County, Silver City, 1 September 1972, ean trap in piñon juniper, 1♀, M.H. Muma, FSCA.7. *New Mexico*, Grant County, Silver City, 16 August 1973, 1♀, 1♂, M.H. Muma, FSCA.5. *New Mexico*, Grant County, Silver City, 3 December 1973, 1♀, M.H. Muma, FSCA.8. *New Mexico*, Grant County, Silver City, 1 September 1973, 4♂, M.H. Muma, FSCA.6. *New Mexico*, Los Alamos County, Los Alamos, R Site Control, August–November 1976, pitfall trap, 1♂, D. Clowns, AMNH. *New Mexico*, Luna County, Nimbres Mtns., Rock Creek Camp, 32.83°N, 107.78°W, 7 September 1941, 3♀, W. Ivie, AMNH. *New Mexico*, Sierra County, 3 km west of Hillsboro, 32.93°N, 107.67°W, 6 September 1941, 1♀, W. Ivie, AMNH. *New Mexico*, Socorro County, south of Magdalena, 34.07°N, 107.27°W, 5 September 1941, 2 vials of 4♀ each, W. Ivie, AMNH. *New Mexico*, Socorro County, west of Socorro, 34.07°N, 106.97°W, 6 September 1941, 2♀, W. Ivie, AMNH. *New Mexico*, Valencia County, Bluewater Lake, 3 km SW of Bluewater Station, 35.28°N, 108.03°W, 4 September 1941, 2 vials of 4♀ each, W. Ivie, AMNH. *New Mexico*, Valeneia County, west of Laguna, 35.07°N, 107.47° W, 5 September 1941, 3 vials with 4♀ each, 1 vial with 12♀, W. Ivie, AMNH. *Texas*, McCullough County, 60 km west of Brady, 31°N, 99°W, December 1939, 1♀, D. & S. Mulaik, AMNH. *Texas*, Oldham County, Adrian, 35°N, 104°W, 4 September 1933, 1♀, W. Ivie, AMNH.

Agelenopsis naevia (Walckenaer 1841)
Ageleua naevia Walckenaer 1841: 24.
Figs. 21, 22, 33, 48, 61A

Type specimens.—Holotype presumed missing. Type locality: *Georgia*.

Etymology.—None given, but "*naevus*" in Latin means pigmented, freckled, birthmark, perhaps indicative of the speckling along the abdominal sides, both in this species and the genus in general.

Diagnosis.—The male of this species can be separated from others in the genus by its loosely coiled embolus, making more than one full cirele, and procurved, tapering tip (Figs. 21, 22). The spermatheca of the female sits well back ventrally of the bursa near the atrium and is only one of two species with a thick and short epigynal bridge (Figs. 33, 48).

Description.—General description as for the genus. Male (n = 26): overall length 12.06–16.73 mm; carapace width at its

widest point 3.82–5.12 mm; carapace width at its narrowest point 2.13–3.05 mm. Shape of RTA is nipple–like; shape of median apophysis is sharp; conductor size is large with generally pointed shape; coiling of embolus is loose with an embolic tip termination angle of 470°; embolus subtriangular segment is absent; embolic tip orientation is not recurved; embolic tip is slender and tapering; anneli on subtegulum are present (Figs. 21, 22). Female (n = 23): overall length 10.52–18.71 mm; carapace width at its widest point 3.52–5.49 mm; carapace width at its narrowest point 2.10–3.12 mm. Bursa is longer than wide, oriented with tips angled slightly dorsally, and narrowly spaced or touching other bursa distally but more widely spaced at base; spermatheca is heart shaped) with conducting tube entering in center of front (seems to diverge and then converge or tubes run parallel); spermathecae touching, set ventrally of bursae, and oriented longitudinally; two narrow turns of fertilization ducts visible; diverticle is thick, curved, and tubular and larger than spermatheca, narrowly separated from other; anterior atrial edge monolobed; epigynal bridge thick and short; bursa opening mostly visible (Figs. 33, 48).

Distribution.—USA: *Alabama*, *Colorado*, *Delaware*, *Florida*, *Illinois*, *Maine*, *Michigan*, *Missouri*, *New Jersey*, *North Carolina*, *Ohio*, *Oklahoma*, *Pennsylvania*, *Tennessee*, *Texas*, *Virginia*, *West Virginia* (Fig. 61A).

Material examined.—USA: *Alabama*, Cleburne County, 9 September 1946, 1♂, H.K. Wallace, FSCA.1232. *Colorado*, Boulder County, 6 November 1960, 1♀, B. Vogel, CU.3. *Colorado*, Boulder County, 2 July 1963, 1♂, G. Patzer, CU.2. *Colorado*, Boulder County, 22 July 1963, 1♂, W.A. Weber, CU.1. *Colorado*, Boulder County, 24 July 1975, 1♂, W.L. Weber, CU.4. *Colorado*, Denver County, 17 July 1948, inside culvert near Hogback, 1♂, W. van Riper, CU.5. *Delaware*, New Castle County, Warwick, Lum's State Park, 14 August 1968, on weeds in grass, 2♀, A. Moreton, MCZ.40204. *Florida*, Alachua County, Gainesville, Division of Plant Industry, 3 November 1981, 1♂, G.B. Edwards, FSAC.1. *Florida*, Liberty County, Torreya State Park, 30.33°N, 84.57°W, 18 December 1967, 1♀, W. Ivie, AMNH. *Illinois*, Union County, Giant City State Park, 9 September 1958, 1♂, R. Willey, MCZ.40198. *Maine*, Bethel County, 44.40°N, 70.80°W, no collection date listed, 1♂, AMNH. *Michigan*, Livingston County, E.S. George Reserve, Grid: L–15, 9 August 1951, 1♀, in grass at barn, H.K. Wallace, AMNH. *Michigan*, Livingston County, E.S. George Reserve; Grid: I–6, 24 July 1951, 1♀, H.K. Wallace, AMNH. *Missouri*, Vernon County, Bronaugh, 28 August 1971, farm garage, 1♀, 1♂, D. Lamore, MCZ.40817. *New Jersey*, Bergen County, Ramsey, 41°N, 74°W, 19 August 1934, 1♂, Ramsey, W.J. Gertsch, AMNH. *New Jersey*, Oakland County, 41°N, 74°W, 4 August 1935, 1♀, 1♂, W.J. Gertsch, AMNH. *North Carolina*, McDowell County, Little Switzerland, 23 August 1930, 2♀, 1♂, W.S. Creighton, MCZ.40194. *North Carolina*, Stokes County, Hanging Rock State Park, 16 August 1968, in web on grass, 1♀, A. Moreton, MCZ.40191. *Ohio*, Hocking County, 8 August 1956, 1♀, R.E. Woodruff, AMNH. *Oklahoma*, Comanche County, Fort Sill, East Range near Sitting Bear Creek, 34.65°N, 98.37°W, 9 July 2004, 349 m, 10:00–11:00 hr, look down, 1♀, 1♂, P.E. Cushing, DMNS ZA.7052. *Oklahoma*, Comanche County, Fort Sill, Geronimo's grave site, 34.68°N, 98.37°W, 19 September, 2003, 1♀, P.E. Cushing, DMNS ZA.6867. *Oklahoma*, Comanche County, Fort Sill, East Range, Geronimo's gravesite in Apache cemetery, around gravestones, 34.70°N, 98.37°W, 2 October 2004, 1♀, 358 m, P.E. Cushing, DMNS ZA.7364. *Pennsylvania*, Jamison County, 3 km E Neshaminy Creek, August 1954, 1♀, 1♂, W. Ivie, AMNH. *Tennessee*, Sevier County, 5 km SW of Gaitlinburg, 35.68°N, 83.55°W, 14 October 1965, 1♀, J. & W. Ivie, AMNH. *Texas*, Anderson County, 17–26 July 2002, 1♂, 11 km east of center of Palestine, pitfall with wings, 31.75°N, 95.48°W, pine: 82.7%, J. Yantis, TXAM.2. *Texas*, Bastrop County, Little Sandy Creek, 16 km NW of Bastrop, 28 October 1971, 1♀, B. Vogel, DMNS ZA.160. *Texas*, Houston County, 10 km north of center of Ratcliff, 31.47°N, 95.13°W, 3–12 June 2002, pitfall, pine 74.3%, 4♂, J. Yantis, TXAM.3. *Texas*, Montgomery County, Decker's Prairie near Tomball, 17 August 1958, 1♀, A. Brady, MCZ.40199. *Texas*, Walker County, Huntsville, 1 km w. of Huntsville, 1♂, C.W. Agnew & D.A. Dean, TXAM.1. *Virginia*, Giles County, H.K. Wallace, FSCA.17. *Virginia*, Montgomery County, 20 August 1948, 1152 m, 1♂, H.K. Wallace, AMNH. *Virginia*, Norfolk County, 13 km south of Portsmouth in Dismal Swamp, 2 km south of Hwy 104 on Hwy 17, 18 September 1968, 1♀, 1♂, (no collector listed), MCZ.40043. *West Virginia*, Pocahantas County, Minnehaha Springs, 38 W 79, July 1947, 2♀, 2♂, W. Haller.

Agelenopsis oklahoma (Gertsch 1936)
Agelena oklahoma Gertsch 1936: 12, f. 4–5
Figs. 17, 18, 38, 53, 61A

Type specimens.—Male holotype, female allotype examined. *Oklahoma*, Payne County, Stillwater, C. Smith, 1934, AMNH.

Etymology.—Named for the state from which the species was described.

Diagnosis.—The male of this species can be separated from others in this genus by its loosely coiling embolus, making two full circles and slender, smooth, tapering tip (Figs. 17, 18). The female is distinctive in its very wide atrium, roughly four times as wide as high (Fig. 38).

Description.—General description as for the genus. Male (n = 47): overall length 6.90–12.86 mm; carapace width at its widest point 1.72–4.36 mm; carapace width at its narrowest point 0.98–2.29 mm. Shape of RTA is somewhat pointed; shape of median apophysis is sharp; conductor size is large with generally pointed shape; coiling of embolus is loose with an embolic tip termination angle of 720°; embolus subtriangular segment is absent; embolic tip orientation is recurved; embolic tip is slender and tapering; anneli on subtegulum are present (Figs. 17, 18). Female (n = 45): overall length 8.90–14.86 mm; carapace width at its widest point 2.45–3.83 mm; carapace width at its narrowest point 1.34–2.23 mm. Bursa is longer than wide, narrowly spaced from other distally but widely spaced at base, with tip angled slightly ventrally; fertilization duct makes two turns around base of bursa; spermatheca is tear-shaped, longitudinally positioned, and oriented dorsally of bursa; conducting tube directly enters anterior edge of spermatheca; diverticle is thick, curved, and tubular, larger than spermatheca, and nearly touching other; anterior atrial edge is slightly bilobed; epigynal bridge is thin and wide; bursa opening mostly visible (Figs. 38, 53) .

Distribution.—CANADA: *Alberta*; USA: *Colorado*, *Kansas*, *Minnesota*, *Montana*, *Nebraska*, *North Dakota*, *Oklahoma*, *South Dakota*, *Utah*, *Wisconsin*, *Wyoming* (Fig. 61A).

Material examined.—CANADA: *Alberta,* Medicine Hat, 14 August (no year given), 1♀, 1♂, J.M. Emerton, MCZ.39977. USA: *Colorado*, Delta County, Crawford, Crawford State Park, 4050 Highway 92, 38.71°N, 107.62°W, summer 2000, 1♂, K.L. Kontour, DMNS ZA.11282. *Colorado*, El Paso County, Cheyenne Mountain State Park campsite, 38.72°N, 104.83°W, 9–11 September 2005, 2♀, 3♂, pit traps in meadow, J. Slowik, DMNS ZA.10611. *Colorado*, El Paso County, Mt. Herman Rd. 329, 2 km west of Monument, CO, 39.22°N, 104.88°W, 7 September 2001, under a rock, 13:32 hr, 2104 m, 21° C, 1♂, B. Morrison, DMNS ZA.4785. *Colorado*, Garfield County, Glenwood Springs, 39°N, 107°W, July 1929, 2♂, E. Mayr, AMNH. *Colorado*, Larimer County, Fort Collins, 40°N, 105°W, 22 August 1946, under old post on ground near lake shore, College Lake, 3♂, C.C. Hoff, AMNH. *Colorado*, Larimer County, Fort Collins, 1713 Richards Lake Rd., 40.62°N, 105.05°W, 29–30 August 1999, 1♀, 1♂, P.L. Wall, DMNS ZA.5510. *Colorado*, Larimer County, 1713 Richards Lake Rd., Ft. Collins, 40.46°N, 105.05°W, 9 September 1999, 1♂, P.L. Wall, DMNS ZA.4760. *Colorado*, Larimer, Fort Collins, 1713 Richards Lake Rd., 40.62°N, 105.05°W, 31 September 1999, 1♀, P.L. Wall. *Colorado*, Larimer County, Fort Collins, 1713 Richards Lake Rd., 40.62°N, 105.05°W, 12 December 1999, 1♀, P.L. Wall, DMNS ZA.4767. *Colorado*, Larimer County, 1713 Richards Lake Rd., Fort Collins, 40.62°N, 105.21°W, 2001 (no other collection date given), 1♂, P.L. Wall, DMNS ZA.5324. *Colorado*, Larimer County, 1713 Richards Lake Rd., Ft. Collins, 40.62°N, 105.21°W, 19 October 2001, basement shower, 1♂, P.L. Wall, DMNS ZA.5509. *Colorado*, Larimer County, Lon Hagler Reservoir, 40.37°N, 105.15°W, 26 August 1999, 13:00–14:00 hr, lookdown, 1♂, N. Shilodon, DMNS ZA.4788. *Colorado*, Mesa County, Fruita, 127 N. Ash St., 39.16°N, 108.73°W, 20 August 2000, casual, 2♀, 1♂, T. Orr, DMNS ZA.8257. *Colorado*, Mesa County, Fruita, 127 N. Ash St., 39.61°N, 108.73°W, 24 August 2000, 1♂, casual, near kitchen, T. Orr, DMNS ZA.8258. *Colorado*, Mesa County, Fruita, 312 N Mesa St., 39.16°N, 108.73°W, 16 September 2002, 1♂, T. Bloomer, DMNS ZA.5022. *Colorado*, Pueblo County, Beulah Highway, Station 1, 30 August 1964, 1♂, no collector listed. *Colorado*, Teller County, Florissant Fossil Beds National Monument, 38.91°N, 105.29°W, 13 August 2002, 2621 m, lookdown, 1♀, 1♂, T. Schroeder & B. Crockett, DMNS ZA.4957. *Colorado*, Weld County, Pawnee National Grasslands, CPER, 11 August 1994, 1♀, 1♂, R.D. Weeks, Jr., USNM.D3. *Colorado*, Weld County, Pawnee National Grassland, off CR 63, 40.70°N, 104.47°W, 21 August 1999, 2♀, 2♂, M. & L. Siderhurst, DMNS ZA.9039. *Colorado*, Weld County, Pawnee National Grasslands, 40.45°N, 103.48°W, 1342 m, 07:00 hr, 16 August 2000, casual, 1♀, 1♂, T. Hiester, DMNS ZA.8259. *Colorado*, Weld County, Bones Galore Paleo Site, 40.73°N, 103.80°W, 12 August 2001, look down, 4.5 hour cum, 16:00–20:30 hr, 1434 m, 1♀, T. Heister, DMNS ZA.9332. *Colorado*, Weld County, Bones Galore Paleo Site, 13–22 August 2000, 1♀, S. & A. Alvarez, DMNS ZA.11838. *Colorado*, Weld County, Bones Galore Paleo Site, 40.73°N, 103.80, 16 August 2001, 1433 m, 17:00–22:00 hr; lookdown, area wetter than last year, 1♂, T. Hiester, DMNS ZA.8260. *Colorado*, Weld County, Erie, 1291 Co Rd 11, 40.02°N, 104.96°W, 1♂, J. Goltl, DMNS ZA.9330. *Kansas*, Calista County, Kingman Lake Park, 13 October 1963, 1♀, 37.39°N, 98.15°W, J. & W. Ivie, AMNH. *Kansas,* Douglas County, Lawrenee, 17 September 1988, at night, wandering on side of trailer, 1♂, H. Guarisco, AMNH. *Kansas*, Russell County, 38.88°N, 98.88°W, 23 August 1935, 3♀, 4♂, W. Ivie, AMNH. *Minnesota*, Rock County, Luverne, 13–14 September 1935, 1♀, 1♂, 43°N, 96°W, Telford (no first initial), AMNH. *Montana*, Gallitin County, Three Forks, 17 August 1941, 2♀, 1♂, 45.87°N, 111.52°W, D. & S. Mulaik, AMNH. *Nebraska*, Sioux County, Oglala National Grasslands, Sugarloaf, 1–12 August 2004, 1♂, shed, H. Guarisco, DMNS ZA.12001. *North Dakota*, Pennington County, 8 km S of Rapid City, 19 September 1950, 1♂, 44°N, 103°W, V. Roth, AMNH. *Oklahoma*, Cleveland County, 15 September–15 November, 1♀, no other collection data given, J. Ward, MCZ.39979. *Oklahoma*, Comanche County, Fort Sill, Quanah, 5 km W of Falcon Gate, 2 October 2004, 1♀, 34.64°N, 98.75°W, 2179 km, 16:20–17:50 hr, look down, P.E. Cushing, DMNS ZA.7366. *Oklahoma*, Payne County, Stillwater, 1934 (no other collection date given), 1♀, 1♂, C. Smith, AMNH. *South Dakota*, Custer County, Beaver Creek Spring, 5 km E of Pringle, 7 August 1954, 1♂, H. & L. Levi, MCZ.39983. *South Dakota*, Kingsbury County, Lake Preston area (formerly Iroquois State Park), 18 September 1950, 10♀, 44°N, 97°W, V. Roth, AMNH. *Utah*, Duchesne County, 40.17°N, 110.4°W, 25 August 1935, 3 vials containing in total 9♀, 3♂, W. Ivie, AMNH. *Utah*, Roosevelt County, 25 August 1935, 1♀, 2♂, 40°N, 110°W, in grass by marsh, W. Ivie, AMNH. *Wisconsin*, Crawford County, Prairie du Chien, October 1950, 197 m, 1♂, M. Melanie, MCZ.39984. *Wyoming*, [Glamos], 24 August 1961, 1♀, R. Lourgue, AMNH.

Agelenopsis oregonensis (Chamberlin & Ivie 1935)
Agelenopsis oregonensis Chamberlin & Ivie 1935: 32, pl. 14, f.108.
Figs. 11, 12, 37, 52, 61B

Type specimens.—Male holotype examined: *Oregon*, Multnomah County, Latourell Falls, Columbia River, 45.55°N, 122.2° W, 4 August, 1929, AMNH. 1♀, 1♂ paratype examined: *Oregon*, Forest Grove County 45°N, 123°W, 6 August 1929, R.V. Chamberlin, AMNH. One female paratype from *Oregon*, Hood River County, Hood River, 4 August, 1929, R.V, Chamberlin, AMNH.

Etymology.—Named for the state from which the species was described.

Diagnosis.—The male of this species is easily distinguished from others in the genus by its slightly tight, then loosely coiling, embolus, making more than one full circle, with a slightly procurved, twisting, and somewhat tapered tip (Figs. 11, 12). The female is distinctive in its spermatheca and diverticle being of equal size, both bulbous, and clearly visible from the ventral side (Fig. 52).

Description.—General description as for the genus. Male (n = 34): overall length 7.45–9.65 mm; carapace width at its widest point 2.60–3.32 mm; carapace width at its narrowest point 1.44–1.79 mm. Shape of RTA is nipple–like; shape of median apophysis is somewhat pointed; conductor size is large with generally pointed shape; coiling of embolus is slightly tight, then loose with an embolic tip termination angle of 540°; embolus subtriangular segment is absent; embolic tip orienta-

tion is not recurved; embolic tip is slender and tapering; anneli on subtegulum are present (Figs. 11, 12). Female (n = 38): overall length 7.95–11.55 mm; carapace width at its widest point 1.70–3.87 mm; carapace width at its narrowest point 0.99–1.89 mm. Bursa is longer than wide, touching other bursa distally but widely spaced at base, oriented vertically with tip angled slightly dorsally; fertilization duct wraps two times around base of bursa; ventral side spermatheca is a longitudinally positioned, tube-shaped structure with conducting tube entering laterally (there is almost no distinction between conducting tube and spermatheca on dorsal side); spermatheca sits in between bursae; diverticle is bulbous and about the same size as spermatheca, with distance between it and other being more than half the diameter of diverticle; anterior atrial edge is distinctly bilobed; epigynal bridge shape is thick and short; bursa opening is mostly visible (Figs. 37, 52).

Distribution.—CANADA: *Alberta*, *British Columbia*; USA: *California*, *Oregon*, *Washington* (Fig. 61B).

Material examined.—CANADA: *Alberta*, July 1927, no other collection information provided, 1♀, O. Bryant, USNM.2031393. *British Columbia*, Wellington, Vancouver Island, 20–24 August 1950, 49°N, 123°W, 2♀, R. Guppy, AMNH. *British Columbia*, Vancouver Island, DND Rocky Point, 22 August 1994, malaise trap, garry oak, forest floor, 1♂, N. Winchester, DMNS ZA.31364. *British Columbia*, Vancouver Island, DND Rocky Point, 24 August 1994, garry oak, malaise trap, open meadow, 1♂, N. Winchester, DMNS ZA.31360. *British Columbia*, Vancouver Island, DND Rocky Point, 26 September 1994, 1♂, N. Winchester, garry oak, open meadow, malaise trap, D. Buckle, DMNS ZA.31362. *British Columbia*, Vancouver Island, DND Rocky Point, 31 July 1995, malaise trap, garry oak, forest floor, 1♀, 2♂, N. Winchester, DMNS ZA.31361. *British Columbia*, Vancouver Island, DND Rocky Point, 26 August 1995, malaise trap, garry oak, open meadow, 1♂, N. Winchester, DMNS ZA.31363. *British Columbia*, Vancouver Island, DND Rocky Point, 26 August 1995, malaise trap, garry oak, forest floor, N. Winchester, 5♂, DMNS ZA.31359. *British Columbia*, Vancouver Island, DND Rocky Point, 4 October 1995, malaise trap, garry, oak forest floor, 1♀, 7♂, N. Winchester, DMNS ZA.31410. USA: *California*, Humboldt County, 8 km SW Orleans, 41°N, 123°W, 22 August 1959, 1♀, W.J. Gertsch & V. Roth, AMNH. *California*, Humboldt County, 3 km N of Phillipsville, 40°N, 123°W, 14 September 1961, 5♀, W.J. Gertsch & W. Ivie, AMNH. *California*, Humboldt County, 29 km west of Willow Creek, 41°N, 123°W, 21 August 1959, 1♀, W.J. Gertsch & V. Roth, AMNH. *California*, Humboldt County, 32 km west Willow Creek, 20 July 1962, 1♀, V. Roth, AMNH. *Oregon*, Jackson County, Ashland, Lithia Park, 42°N, 122W°, 1♀, W.J. Gertsch & V. Roth, 31 August 1959. *Oregon*, Jackson County, Union Creek, 42°N, 122°W, 1006 km, 1–15 September 1950, 3♀, B. Malkin, AMNH. *Oregon*, Yamhill County, Corvallis, MacDonald Forest, 44N°, 123°W, 30 September 1950, 1♀, V. Roth, AMNH. *Washington*, Chelan County, Peavine Creek, 47.86°N, 120.95°W, 22 June 1985, 671 m, web on ground, 1♂, R. Crawford, BMNHC. *Washington*, Clallam County, Olympic National Forest, Mt. Angele, September 1927, 1♂, E. Prye, MCZ.39970. *Washington*, Clallam County, lower Elwha River, Olympic National Park, 15 July 1951, 1♂, H. & L. Levi, MCZ.39980. *Washington*, King County, Seattle, 6–9 August 1935, sweeping vegetation, 1♀, 1♂, E. Sanders, MCZ.39975. *Washington*, King County, Seattle, University of Washington Campus, 47.66°N, 122.31°W, 13 October 1972, in outside stairwell, 61 m, 1♂, R. Crawford, BMNHC. *Washington*, King County, Seattle, University of Washington Campus, 47.66°N, 122.31°W, 5 September 1976, in outside stairwell, 61 m, 1♂, R. Crawford, BMNHC. *Washington*, King County, Seattle, University of Washington, 47.66°N, 122.31°W, 2 October 1993, 61 m, on outside wall of museum building, 1♂, R. Crawford, BMNHC. *Washington*, King County, N 47.75°N, 122.18°W, 3 September 1966, Bothell, 3♂, J.R. Thomson, USNM.2031393. *Washington*, Kittitas County, Kachess Lake, 47.28°N, 121.22°W, 19 August 1973, marsh near lake, 719 m, on web in low shrub, 1♂, R. Crawford, BMNHC. *Washington*, Lewis County, Ike Kinswa State Park, 46.56°N, 122.53°W, 25 June 1988, 201 m, webs on ground edge wood, 1♀, 1♂, R. Crawford, BMNHC. *Washington*, Olympia County, 47°N, 122°W, 4 August 1929, 2♀, 1♂, H. Exline, AMNH. *Washington*, Olympia County, no collection date provided, 8♀, Nathan Banks Collection, no other info, N. Banks, MCZ.39969. *Washington*, Pierce County, Bonney Lake, 47.19°N, 122.18°W, 6 September 1994, 300 m northeast of lake, 195 m, web in shrub in yard, 1♂, T. Gerding, BMNHC. *Washington*, San Juan County, Friday Harbor, N 48.52–56 W 123.00–04, 5 August 1934, 0–147 m, 1♂, H. Exline, BMNHC. *Washington*, Thurston County, Baker Prairie, 46.84°N, 123.08°W, (no date), 46 m, from web in tree on grassy prairie, 1♂, J. Miller, BMNHC. *Washington*, Whitman County, September (no collection year provided), 4♀, Pullman, W.M. Mann, MCZ.39971.

Agelenopsis pennsylvanica (C.L. Koch 1843)
Agelena pennsylvanica Koch 1843: 111, f. 20–21.
Figs. 3, 4, 30, 45, 61C

Type specimen.—Holotype not examined. Type locality: Pennsylvania, Klug.

Etymology.—Named for state from which the species was described.

Diagnosis.—The male of this species is distinguished from other species in this genus by its tightly coiling embolus, making a full circle with pointed tip positioned perpendicular to cymbium (Figs. 3, 4). The female is distinctive in its skull-shaped bursa, very smooth, rounded atrial opening with spermathecae tending to nestle one above the other rather than positioned side by side (Fig. 45).

Description.—General description as for the genus. Male (n = 26): overall length 7.64–12.82 mm; carapace width at its widest point 2.55–4.50 mm; carapace width at its narrowest point 1.54–2.25 mm. Shape of RTA is nipple-like; shape of median apophysis is thick and somewhat pointed; conductor size is large and claw-like; coiling of embolus is tight with an embolic tip termination angle of 470°; embolus subtriangular segment is present; embolic tip orientation is not recurved; embolic tip is slender and tapering; anneli on subtegulum are present (Figs. 3, 4). Female (n = 27): overall length 9.35–14.00 mm; carapace width at its widest point 2.38–4.88 mm; carapace width at its narrowest point 1.32–2.65 mm. Bursa is very rounded, skull-like in profile, converges toward other but does not touch it, and angles ventrally; two turns of

fertilization ducts; spermatheca is kidney shaped, nestled slightly above other, oriented longitudinally, and positioned dorsally of bursa; conducting tube enters spermatheca on front side directly and ventrally; seem to be two parallel conducting tubes; diverticle is curved and tubular, larger than the spermatheca, and less than half a diverticle's distance from the other; anterior atrial edge is smooth; epigynal bridge shape is thin and wide; bursa opening is mostly visible (Figs. 30, 45).

Distribution.—USA: *Colorado*, *Connecticut*, *Idaho*, *Illinois*, *Kansas*, *Louisiana*, *Massachusetts*, *Michigan*, *North Dakota*, *Ohio*, *Oregon*, *Pennsylvania*, *Tennessee*, *Utah*, *West Virginia* (Fig. 61C).

Material examined.—USA: *Colorado*, Adams County, Northglenn, 10565 Kalamath, 39.89°N, 105.00°W, 1661 m, 6 October 1999, 1♀, D.M. Ennis, DMNS ZA.13362. *Colorado*, Adams County, Thornton, 12225 Clermont St., 39.92°N, 104.93°W, 15 August 1999, look up, 21:30–21:40 hr, in flower garden by side of garage, 1♂, P.M. Reed, DMNS ZA.4979. *Colorado*, Adams County, Thornton, 12225 Clermont St., 39.92°N, 104.93°W, 26 August 1999, 18:30–18:40 hr, look up, inside of garage by the flower garden, 1♂, P.M. Reed, DMNS ZA.11286. *Colorado*, Arapahoe County, Aurora, 835 Memphis St., 39.73°N, 104.86°W, 6 October 1999, 1♀, C.P. McAdams, DMNS ZA.13353. *Colorado*, Boulder County, Longmont, Bowron Place, 40.08°N, 105.19°W, 10 September 2001, 1♂, M. Dolieslager, DMNS ZA.11381. *Colorado*, Denver County, Bluff Lake Park off Moline, 39.76°N, 104.86°W, 13 August 2005, 1♀, S. Hink, DMNS ZA.13354. *Colorado*, Denver County, Denver, 1780 Quebec St., 39.74°N, 104.90°W, 27 August 2000, 1♂, C.E. Ransom, DMNS ZA.4793. *Colorado*, Denver County, Denver, 1570 Dahlia St, 39.69°N, 104.93°W, 29 September 2001, 1♀, S. Alvarez, DMNS ZA.13347. *Colorado*, Denver County, Denver, 5121 East Asbury Ave., 39.73°N, 104.80°W, 5 October 2003, 1♀, G.S. Weeding, DMNS ZA.6260. *Colorado*, Jefferson County, Lakewood, 8665 W 13th Ave., 39.74°N, 105.93°W, 1 October 2001, in house, 1♂ R. Johnson, DMNS ZA.8806. *Colorado*, Jefferson, Lakewood, 1320 Dudley, 39.74°N, 105.09°W, Jefferson, 26 August 2003, in bathtub, 0600 hr, 1♂, J. Oliva–Purdy, DMNS ZA.8805. *Colorado*, Jefferson County, Lakewood, 1320 Dudley, 39.74° N, 105.09°W, 11 September 2003, on towel in birdcage 08:30 hr, 1♂, J. Oliva–Purdy, DMNS ZA.6356. *Colorado*, Jefferson County, Lakewood, Jefferson County Open School, 7655 W 10th Ave., 80215, 39.73° N, 105.08, 14 September 1999, 1♂, A. Weis, DMNS ZA.6670. *Colorado*, Jefferson County, Lakewood, Jefferson County Open School, 7655 W. 10th Ave., 39.73°N, 105.09°W, 3 September 2003, 13:30–14:30 hr, 1♂, B. Bartlett, DMNS ZA.6675. *Colorado*, Jefferson County, Lakewood, Jefferson County Open School, 7655 W 10th Ave., 39.73°N, 105.09°W, 18 September 2003, 1♂, [Judith Miller student], DMNS ZA.6667. *Colorado*, Jefferson County, Lakewood, Jefferson County Open School, 7655 W 10th Ave., 39.73°N, 105.09°W, 9 October 2003, 1♂, H. Grant, DMNS ZA.6661. *Colorado*, Jefferson County, Littleton, 5374 S. Datura, 39.62°N, 105.00°W, October 2003, 1♂, A. Williams, DMNS ZA.6563. *Colorado*, Weld, Erie, 1291 Colorado Rd. 11., 40.02°N, 104.96°W, J. Goltl, DMNS ZA.8779. *Colorado*, Weld County, 13 Pierce, 42714 Weld Co Rd. 37, 40.62°N, 104.72°W, October 1999, 1♀, 1♂, Furman family, DMNS ZA.8753. *Colorado*, Yuma County, Bonny State Park, 39.60°N, 102.22°W, 55 R 43 W section, 3 October 2003, 1124 m, school class, 1♀, J.M. Smith, DMNS ZA.13475. *Colorado*, Yuma County, Bonny State Park, 39.60°N, 102.22°W, 4 Oetober 2003, 1123 m, 55 R 43 W section, school class, 1♀, J.M. Smith, DMNS ZA.13475. *Connecticut*, New Haven County, West Rock Park, 19 September 1964, 1♀, B. & C. Durden, DMNS ZA.140. *Idaho*, Nez Perce County, Lewiston, 46°N, 116°W, 21 October 1936, 2♀, W. Ivie, AMNH. *Idaho*, Payette County, NE Fruitland Spring, 44°N, 116°W, 1937, 1♀ , W. Ivie. *Idaho*, Payette County, NE Fruitland Spring, 44°N, 116°W, spring 1942, 1♀, E.M. Ivie, AMNH. *Idaho*, Payette County, NE Fruitland, 44°N, 116°W, fall 1942, 4♀, 1♂, E.M. Ivie, AMNH. *Kansas*, Johnson County, Roeland Park, 25–27 August 1961, 1♂, A.R. Brady, MCZ.40169. *Massachusetts*, Chatham County, Barnstable, 6 September 1976, 1♀, J. Coddington, MCZ.40039. *Massachusetts*, Franklin County, Rowe, 20 August 1965, 2♂, B. Durden et al., DMNS ZA.1891. *Michigan*, Calhoun County, Albion, 12 August 1932, 2♀, 1♂, A.M. Chickering, MCZ.40044. *Michigan*, Calhoun County, Albion, fall 1959, 1♀, 1♂, no collector listed, MCZ.40041. *Oregon*, Yamhill County, McMinnville, 45°N, 123°W, October 1948, 2♀, K.M. Fender, AMNH. *Pennsylvania*, Allegheny County, 24 August 1933, PGH, no other collection information provided, AMNH. *Pennsylvania*, Cambria County, Johnstown, 20 August 1935, 1♂, (no collector listed). *Pennsylvania*, Westmoreland County, 24 August 1935, 1♀, H.K. Wallace, FSCA.456. *Pennsylvania*, York County, Shrewsbury, 1979, 1♀, 1♂, L. Perry, MCZ.40038. *Utah*, Utah County, Canyon Glen in Provo Canyon, September 1942, 1♂, no collector listed, AMNH. *Virginia*, Augusta County, Staunton, 27 August 1970, 1♂, M. Hoffman, MCZ.40166. *Washington*, Walla Walla County, October 1926, 1♀, on porch, no other location information, MCZ.40168. *Washington*, Whitman County, Pullman, September, no day or year listed, W.M. Mann, MCZ.39971.

Agelenopsis potteri (Blackwall 1846)
Agelena potteri Blackwell 1846: 43.
Figs. 5, 6, 31, 46, 61C

Type specimen.—Holotype presumed missing. Type locality: Montreal, Quebec according to Chamberlin & Ivie (1941).

Etymology.—Named for John Blackwall's friend Richard Potter, Esq., M.A., of Queens College, Cambridge, and Professor of Natural Philosophy in University College, London.

Diagnosis.—The male of this species is distinctive in having a tightly coiled embolus, making more than a full circle, with a strongly recurved hooked tip (Figs. 5, 6). The female is distinctive in its bursa folding back ventrally on itself with only its base visible when viewed from the dorsal side and in having a bilobed anterior epigynal edge rather than a smooth one (Figs. 31, 46).

Description.—General description as for the genus. Male (n = 25): overall length 7.06–11.26 mm; carapaee width at its widest point 2.30–4.36 mm; carapace width at its narrowest point 1.37–2.13 mm. Shape of RTA is pointed; shape of median apophysis is thick and somewhat pointed; conductor size is large and claw-like; coiling of embolus is tight with an embolic tip termination angle of 470°; embolus subtriangular

segment is absent; embolic tip orientation is recurved; embolic tip is hooked; anneli on subtegulum are present (Figs. 5, 6). Female (n = 25): overall length 5.75–12.22 mm; carapace width at its widest point 1.96–3.91 mm; carapace width at its narrowest point 1.12–2.11 mm. Bursa is folded back ventrally on itself, giving the appearance of being rounded but is in fact longer than wide, converging in middle ventrally and touching other; two turns of fertilization duct; spermatheca is tear-drop shaped, touches other, and is oriented longitudinally between bursae; conducting tube diverges from other but enters spermathecae directly and anteriorly; diverticle is curved, thick, and tubular, larger than the spermatheca, and almost touches other; anterior epigynal edge is generally smooth but can be minimally rippled or very slightly bilobed; epigynal bridge is thin and wide; bursa opening is mostly visible (Figs. 31, 46).

Distribution.—CANADA: *Ontario*, *Saskatchewan*; USA: *Colorado*, *Indiana*, *Iowa*, *Maine*, *Massachusetts*, *Michigan*, *Minnesota*, *Montana*, *Nebraska*, *North Dakota*, *Washington*, *Wisconsin*, *Wyoming* (Fig. 61C).

Material examined.—CANADA: *British Columbia*, Grand Forks, 1 September 1965, 1♂, J. & W. Ivie, AMNH. *Ontario*, Toronto, Swansea, July 1937, 1♂, H.S. Parish, MCZ.40178. USA: *Colorado*, Adams County, Aurora, 895 Oswego St., 39.73°N, 104.85°W, no collection date listed, 1♀, A. Tucker, DMNS ZA.4982. *Colorado*, Adams County, Northglenn, 10565 Kalamath, 39.89°N, 105.00°W, 12 October 1999, 1661 m, 1 min. looking level, 1♀, D.M. Ennis, DMNS ZA.4794. *Colorado*, Jefferson County, Golden, 835 Urban St., 39.73°N, 105.13°W, 12 September 1999, 20:30–21:30 hr lookdown, 1♀, S. Snover, DMNS ZA.6663. *Colorado*, Jefferson County, Lakewood, 6298 W. 26th Ave., 9 November 1999, 39.75°N, 105.07°W, 1♀, D. Lynch, DMNS ZA.4852. *Colorado*, Jefferson County, Lakewood, Jefferson County Open School, 7655 W. 10th Ave., 39.73°N, 105.08°W, 23 September 1999, 1♀, H. Tran, DMNS ZA.4959. *Colorado*, Jefferson County, Lakewood, Jefferson County Open School, 7655 W 10th Ave., 39.73°N, 105.08°W, 29 September 1999, 1♀, J.L. Kiefert, DMNS ZA.8155. *Colorado*, Jefferson County, Littleton, Chatfield State Park, off C470 & Highway 121, 39.52°N, 105.07°W, 18 August 2001, under bark, 13:58–15:30 hr, 1599 m, 33 °C, 1♀, 2♂, B. Morrison, DMNS ZA.6673. *Colorado*, Jefferson County, Chatfield State Park, Audubon Center Building, 10 September 2004, 39.49°N, 105.09°W, 1689 m, short grass, 2♀, H. Guarisco, DMNS ZA.8641. *Colorado*, Jefferson County, Littleton, W. Chatfield State Park, Highway 121 & C470, 39.52°N, 105.08°W, 13 September 2004, 14.55 hr, 1713 m, in its web, plains, marsh near gravel pond, 1♀, B. Morrison, DMNS ZA.8157. *Colorado*, Jefferson County, Littleton, Chatfield State Park, Highway 121 & C 470, 39.52°N, 105.08°W, 13 September 2004, 1713 m, 14:50 hr, lookdown, under bark near gravel pond, 1♂, B. Morrison, DMNS ZA.8163. *Colorado*, Montezuma County, Mesa Verde National Park, 18 July 2001, casual, piñon and juniper, under rocks and logs, webs in trees, 1♀, A.R. Nabors, DMNS ZA.4768. *Indiana*, Adams County, Decatur, 15 September 1969, on house, 1♀, M. Davis, MCZ.40179. *Iowa*, Cerro Gordo County, Clear Lake, 16 August 1956, 1♀, 3♂, H. & L. Levi, MCZ.40172. *Iowa*, Winnebago County, 22 August 1933, 1♂, I. V. Cantrell, AMNH. *Maine*, Cumberland County, Portland, no collection date provided, 1♀, 1♂, no collector listed, MCZ.40181. *Maine*, Waldoboro County, no collection date listed, E. Lloyd, MCZ.40174. *Massachusetts*, Middlesex County, Pepperell, September 1978, 2♀, 2♂, H., L. & F. Levi, MCZ.40171. *Massachusetts*, Norfolk County, Milton, 28 August–6 September 1956, 2♀, 6♂, H. & L. Levi, MCZ.40175. *Michigan*, Chippewa County, Sault Ste Marie, Sherman Park, 2 September 1980, 2♂, A. Matelski, NMSU. *Minnesota*, Beltrami County, NE Lake Bemidji, 47.52°N, 94.82°W, 10 September 2004, 46 m, in house shingles, 21:00–21:30 hr, lookdown, 3♂, M. Francis, DMNS ZA.6963. *Minnesota*, Ramsey County, St. Paul, University of Minnesota campus, 16 September 1970, in manure pile, 1♀, J. Milne, AMNH. *Nebraska*, Buffalo County, Amherst, October 1971, 1♀, L. Alexander, MCZ.40180. *North Dakota*, Cass County, Fargo, 46.85°N, 96.80°W, no collection date listed, 2♀, W. Ivie, AMNH. *North Dakota*, Cass County, Fargo, 23 August 1965, 1♂, J. Donat, MCZ.40177. *Washington*, Glacier County, Mount Baker, 48.53°N, 121.57°W, 7 September 1965, 1♀, W. & J. Ivie, AMNH. *Wiscousin*, Rock County, Edgerton, 817 County Highway M, 42.79°N, 89.02°W, 24 September 2005, 250 m, house at farm, 1♀, K.M. Potter, DMNS ZA.31941.

Agelenopsis spatula Chamberlin & Ivie 1935

Agelenopsis spatula Chamberlin & Ivie 1935: 32, pl. 14, f. 109.

Figs. 13, 14, 35, 50, 61D

Type specimens.—Male holotype, female allotype, examined; female paratype not examined. *Texas*, Wichita Falls, 33.88° N, 98.45°W, 3 September 1933. W. Ivie, AMNH.

Etymology.—Named for the male's spatulate embolic tip.

Diagnosis.—The male of this species is distinctive in having a somewhat tight coiling of the embolus with a spatulate embolic tip (Figs. 13, 14). The female *A. spatula* is unique in having a thickened diverticle and is one of only two species that has an atrial opening with a monolobed anterior edge (Figs. 35, 50).

Description.—General description as for the genus. Male (n = 11): overall length 8.45–13.24 mm; carapace width at its widest point 2.96–4.53 mm; carapace width at its narrowest point 1.48–2.30 mm. Shape of RTA is nipple–like; shape of median apophysis is thick and somewhat pointed; eonductor size is large and pointed; coiling of embolus is tight with an embolic tip termination angle of 470°; embolus subtriangular segment is present; embolic tip orientation is not recurved; embolic tip is hooked; anneli on subtegulum are present (Figs. 13, 14). Female (n = 18): overall length 7.87–15.49 mm; carapace width at its widest point 2.80–3.84 mm; carapace width at its narrowest point 1.68–2.08 mm. Bursa is longer than wide and somewhat twisted and converging toward other one ventrally; bursae tips are oriented ventrally but not touching; fertilization ducts wind twice around base of bursa; spermatheca is kidney shaped, touching at posterior (ventral) end but diverging radically, positioned longitudinally, sitting generally centrally between bursa; conducting tube enters at front edge so conducting tube angles laterally/diverging ectally; diverticle is curved and tubular, larger than spermatheca, and not touching the other; anterior atrial edge is monolobed; epigynal bridge shape is thin and wide; bursa opening is mostly visible (Figs. 35, 50).

Distribution.—USA: *Colorado*, *Kansas, New Mexico*, *Texas* (Fig. 61D).

Material examined.—USA: *Colorado*, El Paso County, Cheyenne Mountain State Park, lower gate, 38.72°N, 104.82°W, 1886 m, 1♀, J. Slowik, DMNS ZA.10630. *Kansas*, Meade County, Cimarron River, 37.02°N, 100.28°W, 13 August 1964, 1♀, H.S. Fitch, AMNH. *New Mexico*, Quay County, South Tucumcari, 14 August 1990, collected in house, 1♂, S. Moore, NSMU. *New Mexico*, Santa Fe County, 6 km west of Pojoaque, 18 August 1973, 1♂, B. Vogel, DMNS ZA.153. *Texas*, Archer County, Lake Kickapoo, 6 km southeast of Blackflat, 33°N, 98°W, 29 October 1964, 3♀, 1♂, K.W. Haller, AMNH. *Texas*, Clay County, 10 October 2006, collected in rocks near water, 1♂, C. Churchill, MWSU. *Texas*, Dallam County, 2 km east of the Texas line near Colorado border near railroad track, 1452 m, off Hwy 87, 36.39°N, 103.04°W, 2♀, P.E. Cushing, DMNS ZA.7365. *Texas*, Erath County, Stephenville, 7 October 1983, from peanuts, 1♂, C.W. Agnew, TXAM.582. *Texas*, Erath County, 11 km northeast of Stephenville, 17 May 1983, 1♂, C.W. Agnew, TXAM. *Texas*, Frio County, Pearsall, 18 October 1939, 1♂, C.E. Heard, AMNH. *Texas*, Houston County, 3 km southwest of center of Kennard, 22–31 May 2001, 1♀, 31.33°N, 95.22°W, pine 88.1%, pitfall original plot 38, J. Yantis, TXAM.02. *Texas*, Roberts County, 16 September 1972, roeks, 1♀, T. Kaspar, MWSU. *Texas*, Travis County, Austin, 5 October 1968, 1♂, B. Vogel, DMNS ZA.1899. *Texas*, Wichita County, 6 km southwest of Burkburwett, 25 October 1975, web in rocks cross tanks, 1♀, J. Cokendolpher, MWSU. *Texas*, Wichita County, 8 November 1980, on ground, 1♀, F. Stangel, MWSU. *Texas*, Wichita County, 8 February 1995, on ground, 1♀, D. Reddick, MWSU. *Texas*, Wichita County, 27 February 1995, under rock, 1♀, D. Reddick, MWSU. *Texas*, Wichita County, 30 September 2001, pitfall, 1♂, C.J. Bowen, Jr., MWSU. *Texas*, Wichita County, 23 October 2001, pitfall, 1♂, C.J. Bowen, Jr., MWSU. *Texas*, Wichita County, 30 October 2001, pitfall, 1♀, C.J. Bowen, Jr., MWSU. *Texas*, Williamson County, Florence, 3 km east of N. Salado Creek, 23 October 1971, 1♀, B. Vogel, DMNS ZA.13479. *Texas*, Williamson County, 4 km northwest of Jarrell, 23 October 1971, 3♀, B. Vogel, DMNS ZA.156.

Agelenopsis utahana (Chamberlin & Ivie 1933)
Agelena utahana Chamberlin & Ivie 1933: 43, pl. 11, f. 113–115.
Figs. 9, 10, 36, 51, 61B

Type specimens.—Male holotype and female allotype examined. *Utah*, Box Elder County, Raft River Mountains, Clear Creek, 4 September 1932, 41.95°N, 113.17°W, Ivie, AMNH 1♂, 4♀ paratypes examined: *Utah*, Utah County, Aspen Grove, 40°N, 111°W, August 1926, Harris & Tanner, AMNH. 1♂, 3♀ paratypes examined: *Utah*, Utah County, 32°E, 40°N, 111°W, 675 ft (205.7 m), AMNH.

Etymology.—Named for state from which the species was described.

Diagnosis.—The male of this species is distinctive for its very tightly coiled embolus with slender, procurved tip (Figs. 9, 10). The female of this species is distinctive for being only one of two species in this genus with a thick and short epigynal bridge shape, along with *A. naevia* (Fig. 36).

Description.—General description as for the genus. Male (n = 25): overall length 6.42–9.04 mm; carapace width at its widest point 2.35–3.26 mm; carapace width at its narrowest point 1.25–1.80 mm. Shape of RTA is nipple–like; shape of median apophysis is blunt; conductor size is large and truncate; coiling of embolus is tight with an embolic tip termination angle of 470°; embolus subtriangular segment is absent; embolic tip orientation is not recurved; embolic tip is slender and tapering; anneli on subtegulum are present (Figs. 9, 10). Female (n = 25): overall length 6.10–10.92 mm; carapace width at its widest point 1.99–3.32 mm; carapace width at its narrowest point 1.07–1.95 mm. Bursa is longer than wide, widely spaced from the other, and angled slightly ventrally from vertical; fertilization ducts coil twice around the base of the bursa, spermatheca is more knob-like at distal end of connecting tube than rounded itself and is oriented transversely, positioned prominently on the ventral side; conducting tubes seem to be looping around entrance to spermathecae, entering anteriorly; diverticle is small, bulbous (about same size as spermatheca), heavily sclerotized, rounded, and widely set from other; anterior edge of atrium is distinctly bilobed; epigynal bridge is thick and short; copulatory duct opening is mostly visible (Figs. 36, 51).

Distribution.—CANADA: *Alberta*, *British Columbia*, *Manitoba*, *Saskatchewan*; USA: *Alaska*, *Colorado*, *Massachusetts*, *Michigan*, *Montana*, *New Hampshire*, *New York*, *Ohio*, *Pennsylvania*, *Utah*, *Virginia*, *Wyoming* (Fig. 61B).

Material examined.—CANADA: *Alberta*, 29 July 1949, 1♀, [Conklin], AMNH. USA: *Alaska*, North Star Borough, Southeast Fairbanks, 3 July 2000, 1♀, 1♂, F. Levi, MCZ.21927. *Colorado*, Denver County, Denver, 535 Dahlia, 39.72°N, 104.93°W, 11 August 2003, 2♂, J.I. Gilman, DMNS ZA.8449. *Colorado*, Gilpin County, Golden Gate Canyon State Park, Bootleggers Bottom Trail, 39.86°N, 105.45°W, 3 August 2001, 2717 m, 15:00–16:00 hr, sweep/look down, creek bed ravine, 1♀, K. Egerman, DMNS ZA.8263. *Colorado*, Jefferson County, Golden Gate Canyon State Park, Ranch Ponds, 39.85°N, 105.37°W, 11 August 2002, 2377 m, 12:40–14:00 hr, lookdown, 1♂, P.E. Cushing, DMNS ZA.11461. *Colorado*, Jefferson County, Elk Meadow Open Space Park, Stagecoach Blvd., 13:00–15:00 hr, 1♀, K. P. Owens, DMNS ZA.8261. *Colorado*, Larimer County, Drake, Roosevelt National Forest on Waltonia River, 40.41°N, 105.36°W, in cabin shed, 8 July 2001, 1♀, M. Spoon, DMNS ZA.6665. *Colorado*, Las Animas County, Bar NI Ranch, 37.16°N, 105.37°W, 14 July 2001, during Bioblitz, 2984 m, lookdown, 1♂, D.W. James, DMNS ZA.11919. *Colorado*, Montezuma County, Mesa Verde National Park, campground, 37.30°N, 108.42°W, 14–16 August 2005, 2316 m, casual camp spiders, 2♂, P.E. Cushing, J.T. Stephenson, T. Heister, J. Slowik, DMNS ZA.11387. *Maine*, Piscataquis County, 1.9 km south–southeast of Soubunge Mountain., Line I, Tn. 2 T4 R11, 28 July 1977, WELS spruce–fir forest; stripcut, pitfall, 1♂, M.W. Houseweart & D.T. Jennings, AMNH. *Michigan*, Livingston County, ES George Reserve, grid P–18, 24 June 1951, 1♂, H.E. Wallace, AMNH, vial #1421. *Montana*, Carbon County, East Rosebud Lake, 45°N, 109°W, 1890 m, 6 July 1966, 1♂, B. Vogel, DMNS ZA.144. *Montana*, Carbon County, E. Rosebud Lake, 14 August 1967, 1890 m, B. & C. Durden, DMNS ZA.148. *Montana*, Carbon County, Rosebud Canyon, 17 August 1967, 1920 m, 1♂, B. & C. Durden, DMNS ZA.151. *Montana*, Carbon County, E. Rosebud Lake, 25 August 1977,

1890 m, 1♀, 1♂, B. & M. Vogel, DMNS ZA.13482. *Montana*, Flathead County, Bigfork, 23–24 August 1957, 914 m, field w. stones & logs, 2♀, 2♂, H. & L. Levi, MCZ.40025. *New Hampshire*, Carroll County, Moultonboro, Kona Bay, 9 August 1980, woods, 3♀, 2♂, H. & L. Levi, MCZ.40021. *New Hampshire*, Grafton County, Franconia, 44.22°N, 71.73°W, no collection date listed, no collector listed, 1♀, 1♂, AMNH. *New York*, Sullivan County, Beaver Kill, 41°N, 74°W, 30 August 1944, 1♂, R.B. Fisher, AMNH. *New York*, Ulster County, Chichester, 42°N, 74°W, 3 August 1945, 2♂, T. Cohn, AMNH. *New York*, Ulster County, Chichester, 42°N, 74°W, 8–21 August 1945, 1♀, 1♂, T. Cohn, AMNH. *Ohio*, Franklin County, Columbus, 28 September 1950, 1♀, H.V. Weems, Jr., FSCA. *Pennsylvania*, Westmoreland County, 5 km south of Rector (PNR), 40.10°N, 79.14°W, 28 August 1966, 2♀, B. Vogel, DMNS ZA.139. *Utah*, Salt Lake County, near mouth of Red Butte Canyon, 6 August 1948, sweeping, 1♀, 2♂, K. Lafferty, AMNH. *Utah*, Utah County, 19 August 1977, on ground, 2♀, J.C. Cokendolpher, MWSU. *Utah*, Utah County, Manti-LaSal National Forest, 16 km west of Monticello, 20 August 1977, 1♀, J.C. Cokendolpher, MWSU. *Virginia*, Giles County, 5 August 1949, Wallace Collection, 2♀, H.K. Wallace, FSCA. *Virginia*, Giles County, Mountain Lake, August 1949, 1♂, H.K. Wallace, FSCA. *Virginia*, County unknown, Poor Man's Mountain, SR 17, 1♂, det. by V. Roth, 1952, FSCA. *Wyoming*, Teton County, woods above lily pond just south of Moran, 43°N, 110°W, 17 August 1950, 2♀, D.C. Lowrie, AMNH.

DISCUSSION

Ayoub et al. (2005) carried out a molecular phylogenetic analysis of various populations of *Agelenopsis* including all the species except *A. actuosa*. In their study, they noted well supported species groups including: *A. aleenae* and *A. spatula*; *A. utahana* and *A. oregonensis*; and a third including *A. potteri, A. pennsylvanica,* and *A. emertoni*. Our morphological cladistic analysis supports the existence of these species groups, demonstrating a close relationship between *A. aleenae* and *A. spatula* as well as a relationship between *A. oregonensis* and *A. utahana,* and a well-supported clade including *A. pennsylvanica*, *A. potteri*, and *A. emertoni*. Our analysis, which included *A. actuosa*, placed this species with the *A. pennsyvanica, A. potteri, A. emertoni* clade: (((*A. pennsylvanica* + *A. potteri*) + *A. actuosa*) + *A. emertoni*). Our analysis suggested that *A. longistyla* is more closely related to the *A. oregonensis* + *A. utahana* clade and we also found that *A. oklahoma* is more distantly related to the clade including *A. oregonensis, A. utahana,* and *A. longistyla* than to the other species. Our 50% consensus tree (Fig. 62) showed > 50% bootstrap support for all clades.

The atrium of both female *A. aleenae* and *A. spatula* are very similar, particularly along the monolobed anterior edge (Figs. 34, 35), as are the spatulate embolic tips of the males of these two species (Figs. 2, 14). *Agelenopsis oregonensis* and *A. utahana* show similarities in the internal genitalia, particularly the shape of the spermatheca and the diverticle (Figs. 51, 52). Both *A. actuosa* and *A. pennsylvanica* have similarities in the shape of the embolic tip (Figs. 4, 8). The subtriangular segment of the embolic tip is also seen in *A. emertoni* (Fig. 26). *Agelenopsis actuosa*, *A. pennsyvanica*, and *A.potteri* all have similarly shaped atrial openings (Figs. 30–32).

Agelenopsis aleenae, *A. spatula*, and *A. aperta* inhabit a narrowly defined area within the southwestern United States (Fig. 61D). *Agelenopsis aperta* resides in all of the southwestern states from California to Texas while *A. aleenae* and *A. spatula* occupy a narrower region in Colorado, New Mexico, and Texas. In an agelenid study in the Malpais Lava Beds in central New Mexico, *A. aleenae* and *A. spatula* were not collected together, but in one instance *A. aperta* was found with *A. spatula* (Ayoub et al. 2005). In none of our collecting trips did we ever find *A. aleenae* and *A. spatula* together although there is great overlap in their respective ranges, suggesting that these two morphologically similar species (as well as other species of the genus) may be segregated by habitat, as suggested by Guarisco (2014). Based on their collection records, Ayoub & Riechert (2004) estimate that the range of *A. aperta* is limited to below 2000 meters and that the species is more susceptible to cold than some other agelenid species. Yet the range of *A. aperta* includes all of Colorado and Utah as well as parts of Wyoming, an area covering elevations well above 2000 meters (Fig. 61D), confirming that this species may well be hardier and more adaptable to a variety of habitats than the comparatively smaller species of *A. aleenae* and *A. spatula* with more restricted ranges.

While none of the other *Agelenopsis* species share both morphological *and* geographical similarities like the group including *A. aleenae* and *A. spatula*, it is noteworthy that the other *Agelenopsis* species with more limited ranges, specifically *Agelenopsis longistyla* (Fig. 61B, circles) and *A. oregonensis* (Fig. 61B, triangles) tend to be small or medium in size (10 mm or less in length) and have shorter maturation periods (from roughly August through September) than larger and more widespread *Agelenopsis* species (Paison 1997). *Agelenopsis longistyla* is located primarily in the southwestern states of Arizona, Colorado, New Mexico, and Texas while the range of *A. oregonensis* is confined to the northwestern portion of North America including northern California, Oregon, and Washington as well as western Canada (Fig. 61B). As with *A. aleenae* and *A. spatula*, these species may be more confined to particular ranges because they may be less adaptive to varying habitats.

Our study provides the first revision of all 13 species in the genus *Agelenopsis*. In addition to *Agelenopsis* monophyly, our cladistic analysis provides support for the species groups proposed by the molecular study of Ayoub et al. (2005). The distinct morphological differentiation of genitalic structures among species in this genus, along with the recognition that *Agelenopsis* species are ground, and most likely not aerial dispersers (Ayoub et al. 2005), suggests that this genus may provide an ideal system to test hypotheses regarding the importance of morphology and habitat segregation in speciation.

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Twelve new Neotropical species of the spider genus *Cryptachaea* (Araneae: Theridiidae)

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Abstract. *Cryptachaea* Archer 1946 currently contains 75 species. The genus is considered related to *Achaearanea* Strand 1929, *Hentziectypus* Archer 1946, and *Parasteatoda* Archer 1946 but differs from them in having a male palp with a short to medium embolus, which is not articulated and not supported on the back of the cymbium; the base is fused to the median apophysis, located in the hood of the cymbium. The *Cryptachaea* epigynum has uncoiled ducts leading to a pair of simple subcircular spermathecae. Twelve new species of *Cryptachaea* are here newly described from South America, seven from Brazil: *Cryptachaea cidae* **new species** from the state of Rio Grande do Sul; *C. alleluia* **new species** from Minas Gerais and Paraná; *C. spectabilis* **new species** from Paraná; *C. divisor* **new species** from the states of Amazonas, Acre, Rio de Janeiro, and Paraná; *C. floresta* **new species** from Amazonas; *C. ericae* **new species** and *C. propinqua* **new species** both from the state of Rio Grande do Sul. *Cryptachaea catita* **new species** occurs in Brazil and Argentina. Two new species are from Peru (*C. tambopata* **new species** and *C. paquisha* **new species**) and two are from Bolivia (*C. benivia* **new species** and *C. lavia* **new species**).

Keywords: Neotropical Region, Argentina, Bolivia, Brazil, Peru

Cryptachaea was first proposed by Archer (1946) as a subgenus of *Theridion* Walckenaer 1805, and was then elevated to genus level (Archer 1950) with *Theridion catapetraeum* Gertseh and Archer 1942 as the type species (= *Cryptachaea porteri* (Banks 1896)). Although Levi (1955) eonsidered the name *Cryptachaea* as a junior synonyn of *Achaearanea* Strand 1929, recently Yoshida (2008) revalidated the genus. Currently, the genus contains 75 species, of which 57 occur in the tropics (World Spider Catalog 2014).

Cryptachaea is closely related to *Achaearanea*, *Hentziectypus* Archer 1946 and *Parasteatoda* Archer 1946 but can be distinguished from these genera by its male palp with a short to medium embolus, which is not articulated and not supported on the back of the eymbium; the base is fused to the median apophysis lodged in the cymbial hood (Levi 1955; Agnarsson 2004; Yoshida 2008; Buckup et al. 2012). The epigynum of *Cryptachaea* has simple, uncoiled ducts leading to a pair of simple subcircular spermathecae and a slightly sclerotized plate. The species of *Cryptachaea* lack the theridiid tegular apophysis, and as other members of the subfamily Theridiinae, also lack a colulus (Agnarsson 2004; Buckup et al. 2012). In this paper we describe and illustrate twelve new species of *Cryptachaea* from Peru, Bolivia, Argentina, and Brazil.

METHODS

Genital terminology follows Levi & Levi (1962), Agnarsson (2004), and Agnarsson et al. (2007). The female genitalia were cleared in lactic acid 85% for an hour, in ambient temperature, allowing the visualization of the internal structures. The illustrations were made with a stereomicroscope with a *camera lucida*, and sometimes a microscope was used for better analysis of the ducts. The measurements are in millimeters (mm).

The individuals examined are deposited in the following institutions (curators in parentheses): IBSP, Instituto Butantan, São Paulo (A.D. Brescovit); INPA, Instituto Nacional de Pesquisas Amazônicas, Manaus (C. Magalhães; A.L. Henriques); MCN, Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre (R. Ott); MUSM, Museu de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima (D. Silva); MLP, Museu de La Plata, La Plata (L.A. Pereira); MPEG, Museu Paraense Emílio Goeldi, Belém (A.B. Bonaldo); NMNH, National Museum of Natural History, Smithsonian Institution, Washington D. C. (J.A. Coddington).

Abbreviations used in figures and text are as follows: AME, anterior median eyes; PME, posterior median eyes; ALE, anterior lateral eyes; PLE, posterior lateral eyes.

TAXONOMY

Family Theridiidae Sundevall 1833
Subfamily Theridiinae Sundevall 1833
Genus *Cryptachaea* Archer 1946
Cryptachaea cidae **new species**
Figs. 1a–c, 3b

Type material.—Holotype: Male (MCN 24450), BRAZIL, *Rio Grande do Sul*, Cambará do Sul, Área de Preservação Ambiental Celulose Cambará, 26.xi.1993, L. Moura leg. Paratype: Female (MCN 28865), São Francisco de Paula, Barragem Passo do Inferno, 19.xi.1997, M.A.L. Marques leg.

Etymology.—The specific name honors the arachnologist Maria Aparecida L. Marques (MCN/FZBRS), nicknamed Cida, in recognition for her many contributions to this manuscript, including some illustrations. The species epithet is a genitive noun.

Diagnosis.—The male palpus of *Cryptachaea cidae* **new species** differs from the other species of the genus by the apex of the cymbium with a conspicuous retrolateral digiform prong (Fig. 1a). The females have a rounded epigynum, as *C. lisei* Buckup, Marques & Rodrigues 2010 (see Buckup et al. 2010, Fig. 11), but differ from this species by the position of

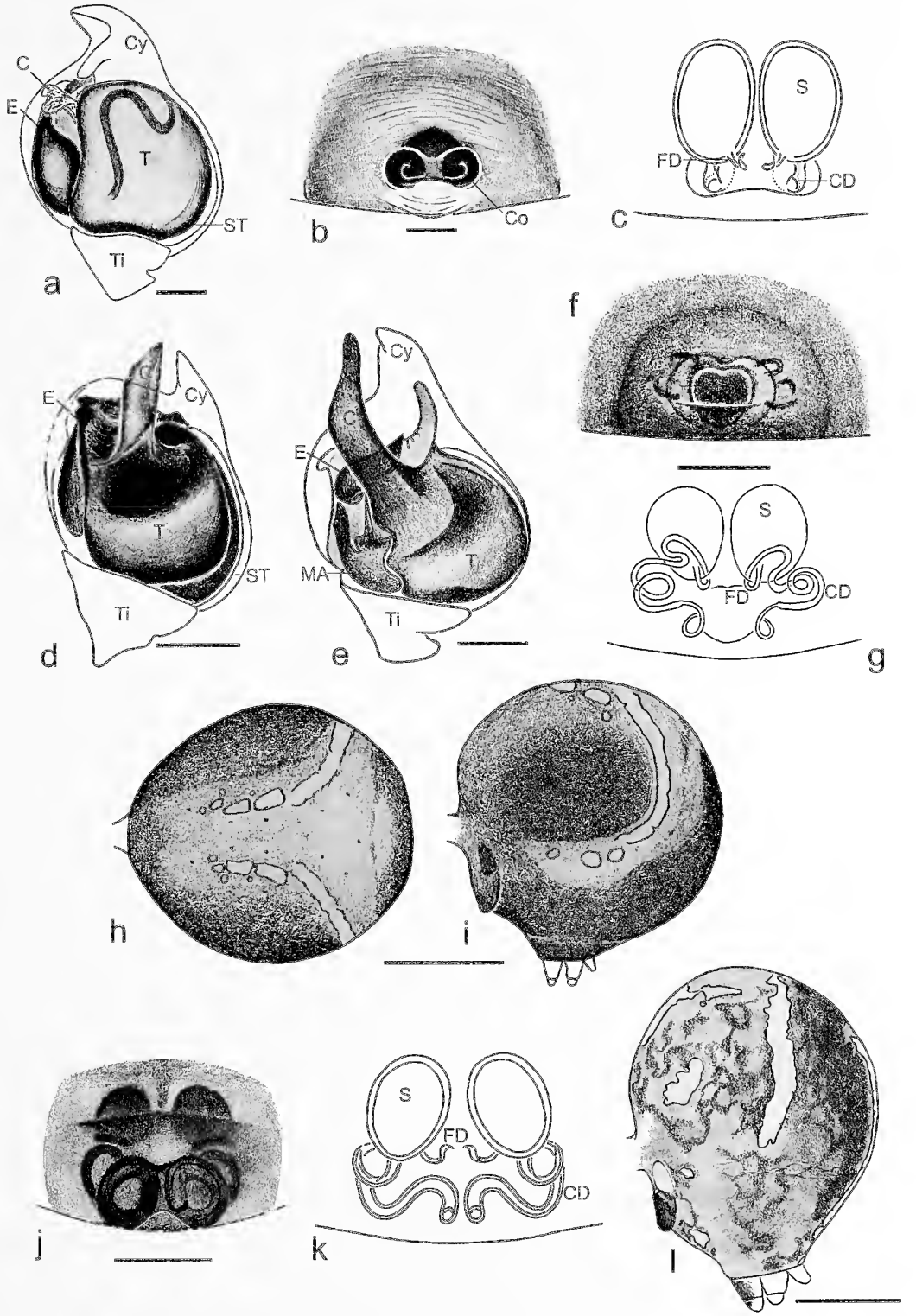

Figure 1a-l.—*Cryptachaea* **new species**. *Cryptachaea cidae* **new species**. a. male palpus, ventral; b. epigynum, ventral; c. epigynum, dorsal. *Cryptachaea alleluia* **new species**. d. male palpus, ventral. *Cryptachaea spectabilis* **new species**. e. male palpus, ventral. *Cryptachaea divisor* **new species**. f. epigynum, ventral; g. epigynum, dorsal; h. female habitus, dorsal; i. female habitus, lateral. *Cryptachaea floresta* **new species**. j. epigynum, ventral; k. epigynum, dorsal; l. female habitus, lateral. Scale bars: a–g, j, k = 0.1 mm; h, i, l = 0.5 mm (C, conductor; CD, copulatory ducts; Cy, cymbium; E, embolus; FD, fertilization ducts; MA, median apophysis; O, copulatory opening; S, spermathecae; ST, subtegulum; T, tegulum; Ti, tibia).

the copulatory openings (Fig. 1b, c), which are facing each other in *C. cidae* **new species** but away from each other in *C. lisei*.

Description.—Male (MCN 24450): Carapace, chelicerae, labium, and endites orange; black pigment between the median eyes. Sternum orange, with a black stain near the coxae I and in the posterior border. Legs orange with gray pigment, more conspicuous on the tibiae. Abdomen longer than high, black, except around the pulmonary area. AME largest, separated from each other by three-quarters of an eye's diameter and located very close to the ALE. Posterior eyes equidistant, separated from each other by the diameter of the PME. Measurements. Total length 2.47. Carapace: length 1.17, width 0.97. Abdomen: length 1.37, width 0.87, high 0.92. Legs formula 1423, length I/II/III/IV: femora 1.67/1.12/0.80/1.27; patellae 0.50/0.42/0.35/0.40; tibiae 1.30/0.75/0.50/0.80; metatarsi 1.55/0.92/0.65/0.87; tarsi 0.65/0.52/0.40/0.52. Total legs length 5.67/3.73/2.70/3.86.

Variation: Total length (n = 2) 2.44–2.50; carapace 1.15–1.18; femur I 1.62–1.72.

Female (MCN 28865): Carapace, chelicerae, labium, and endites orange; black pigment between the median eyes. Sternum yellowish-orange, with a black stain near the coxae I and in the posterior border. Legs orange with gray pigment, which are more conspicuous on the tibiae. Abdomen longer than high, dark brown except around the pulmonary area. Posterior eyes equidistant, separated from each other by PME diameter. AME larger than the other eyes, separated from each other by three-quarters of an eye's diameter and located very close to the ALE. Measurements. Total length 2.82. Carapace: length 1.06, width 0.92. Abdomen: length 1.70, width 1.22, high 1.25. Legs, formula 1423, length I/II/III/IV: femora 1.56/1.02/0.78/1.25; patellae 0.44/0.37/0.30/0.47; tibiae 1.12/0.68/0.51/0.85; metatarsi 1.36/0.81/0.51/0.91; tarsi 0.64/0.54/0.40/0.54. Total legs length: 5.12/3.42/2.50/4.02.

Variation.—Total length (n = 6) 2.80–2.91; carapace 1.04–1.08; femur I 1.56–1.62.

Additional material examined.—BRAZIL, *Rio Grande do Sul*: São Francisco de Paula (Fazenda Três Cachoeiras), ♂, 4♀, 05.xi.1998, A.B. Bonaldo leg. (MCN 30942); Caxias do Sul (Vila Oliva), ♀, 15.i.1974, F.R. Meyer leg. (MCN 2003).

Distribution.—BRAZIL (*Rio Grande do Sul*) (Fig. 3b).

Cryptachaea alleluia **new species**
Figs. 1d, 3a

Type material.—Holotype: Male (MCN 22700), BRAZIL, *Paraná*, Ponta Grossa (Vila Velha), 19.i.1987, Equipe Profaupar leg. Paratype: Male (IBSP 165997), *Minas Gerais*, Alto Caparaó (Parque Nacional do Caparaó), 01–07.v.2002, Equipe Biota leg.

Etymology.—The specific name is a noun in apposition of Hebrew origin (Hallelujah) that means "praise to God," reflecting the accidental loss and subsequent recovery of the type specimen.

Diagnosis.—*Cryptachaea alleluia* **new species** (Fig. 1d) resembles *C. spectabilis* **new species** (Fig. 1e) by the prominent conductor, which can be distinguished in *C. alleluia* **new species** by its laminar shape and pointed distal end and by the shape of the palpal tibia (Fig. 1d).

Description.—Male (MCN 22700). Carapace yellow with gray pigment. Chelicerae dark yellow with gray pigment. Labium, endites, coxae, and sternum pale yellow; sternum with gray spots near the legs. Legs pale yellow; leg I, partially gray ventrally; distal portion of the articles gray, with tarsi almost totally gray. Tibiae IV, with a conspicuous dark-ringed band. Abdomen black with a wide transversal and uncolored band (dorsal view) with stridulatory organs in the dorsal anterior border. Ventrally, there are two circular yellow stains. PME larger than the other eyes, separated from each other by three-quarters of an eye's diameter and placed close to the ALE. The posterior eyes are equidistant, separated from each other by more than half of the PME. Measurements. Total length 1.70. Carapace: length 0.82, width 0.70, high 0.87. Abdomen: length 0.87, width 0.70, high 0.87. Legs, formula 1,2=4,3, length I/II/III/IV: femora 1.12/0.75/0.57/0.80; patellae 0.37/0.32/0.25/0.30; tibiae 0.85/0.52/0.32/0.50; metatarsi 0.87/0.52/0.37/0.52; tarsi 0.47/0.42/0.35/0.40. Total legs length: 3.68/2.53/1.86/2.52.

Female: unknown.

Distribution.—BRAZIL (*Paraná* and *Minas Gerais*)—(Fig. 3a).

Cryptachaea spectabilis **new species**
Figs. 1e, 3a

Type material.—Holotype: Male (MCN 9159), BRAZIL, *Paraná*, Curitiba, 02.xii.1978, A. Yamamoto leg. (Malaise trap).

Etymology.—The specific name is an adjective that means "remarkable," referring to the conspicuous conductor of the male palpus.

Diagnosis.—The male of *Cryptachaea spectabilis* **new species** can be easily distinguished from the other species of the genus by the very developed, sclerotized and digitiform conductor (Fig. 1e). The embolus is short and the median apophysis has two lateral projections, one on each side (Fig. 1e).

Description.—*Male* (MCN 9159). Carapace dark yellow, with median and lateral area slightly gray. Chelicerae yellow, with two teeth in promargin. Labium and endites yellow. Sternum pale yellow, with slightly gray borders. Legs pale yellow with gray pigment. Abdomen, dorsal view, yellow with a discontinuous median-longitudinal dark gray band; anterior and posterior margin of each side dark gray. Ventral view, slightly gray, except for two circular yellow marks. Abdomen spherical, with stridulatory organs anteriorly. AME smaller than the other eyes, separated from each other by almost an eye's diameter and placed very close to the ALE. PME separated from each other by about two-thirds of an eye's diameter and by approximately its radius to the ALE. Measurements. Total length 1.92. Carapace: length 0.85, width (larger width) 0.77. Abdomen length 1.02, width 0.87, high 1.12. Legs, formula 1,2=4,3, length I/II/III/IV: femora 1.25/0.85/0.65/0.92; patellae 0.40/0.35/0.25/0.32; tibiae 0.89/0.57/0.37/0.57; metatarsi 0.97/0.60/0.44/0.57; tarsi 0.55/0.44/0.37/0.42. Total legs length: 4.96/2.81/2.08/2.80.

Female: unknown.

Distribution.—BRAZIL (*Paraná*) (Fig. 3a).

Cryptachaea divisor **new species**
Figs. 1f–i, 3a

Type material.—*Holotype*: Female (IBSP 8966), BRAZIL, *Acre*, Parque Nacional da Serra do Divisor (Piroca), 9.xi.1996,

R.S. Vieira leg. Paratypes: female (MCN 48503), same data as holotype; female (IBSP 9156), (Pedernal), 13.xi.1996, R.S. Vieira leg.; female (INPA), *Amazonas*, Manaus (Igapó, rio Tarumã-Mirim), 08.ii.1988, H. Höfer leg.

Etymology.—The specific name is a noun in apposition taken from the type locality.

Diagnosis.—Females of *Cryptachaea divisor* **new species** resemble those of *C. dalana* (Buckup and Marques 1991) (see Buckup and Marques 1991, Figs. 4–6) by the color pattern of the abdomen (Fig. 1h, i) from which they differ by the unusual subtriangular, heart-shaped atrium (Fig. 1f). Internally, the copulatory duct is characteristieally coiled (Fig. 1g).

Description.—*Female* (MCN 48503). Carapace brown with gray radial grooves. Chelicerae and endites yellow with gray pigment. Palpus, sternum, and labium brownish-gray. Legs brown and yellow. Abdomen spherical, dark brown, dorsally with a pair of narrow longitudinal and discontinuous, laterally diverging white bands (Fig. 1h, i); posterior area with sparse white dots and a vestigial median longitudinal white band; there are two small white marks on each side of the spinnerets. Venter dark gray. Eyes are subequal. AME separated from each other by an eye's diameter, close to the ALE. Posterior eyes equidistant, separated from each other by the PME diamenter. Measurements. Total length 1.85. Carapace: length 0.74, width 0.59. Abdomen: length 1.25, width 1.20, high 1.32. Legs, formula 1423, length I/II/III/IV: femora 0.82/0.67/0.52/0.78; patellae 0.27/0.25/0.21/0.29; tibiae 0.55/0.39/0.25/0.44; metatarsi 0.59/0.42/0.32/0.46; tarsi 0.40/0.36/0.29/0.34. Total legs length: 2.63/2.09/1.59/2.31.

Variation: Total length (n = 6) 1.76–2.03; carapace 0.67–0.74; femora I 0.74–0.94. Some females have the abdomen laterally black and the dorsum with depigmented bands. The trajectory of the genital ducts may vary in specimens.

Male: unknown.

Additional material examined.—BRAZIL, *Amazonas*: Manaus (Igapó, rio Tarumã-Mirim), ♀, 30.vii.1979 (canopy fogging,) J. Adis et al. leg. (INPA); *Rio de Janeiro*: Volta Redonda (Floresta da Cicuta), ♀, 11–18.vi.2001, Equipe Biota leg. (IBSP 85806); *Paraná*: Foz do Iguaçu (Parque Nacional do Iguaçu), 25°36′S, 54°25′W, ♀, 03–12.iii.2002, Equipe Biota leg. (MCN 48504); ♀ (IBSP 85805).

Distribution.—BRAZIL (*Amazonas*, *Acre*, *Rio de Janeiro*, and *Paraná*) (Fig. 3a).

Cryptachaea floresta **new species**
Figs. 1j–l, 3a

Type material.—*Holotype*: Female (MCN 21994), BRAZIL, *Amazonas*, Manaus, Reserva Florestal Adolfo Ducke, 18.xii.1987, E.H. Buckup leg. Paratypes: female (MCN 21995), as in holotype; female (MCN 21293), as in holotype, 15–23.viii.1991, A.D. Brescovit leg.; 3 females (MCTP 8774), as in holotype, 19–24.ii.1992, A.A. Lise & A.B. Bonaldo leg.

Etymology.—The specific name is a noun in apposition that refers to the forested area of the type locality.

Diagnosis.—Females of *Cryptachaea floresta* **new species** (Fig. 1l), resemble those of *Cryptachaea benivia* **new species** by the color pattern, but they differ by the projecting and transparent epigynum with long and wide ducts making two coils (Fig. 1j, k).

Description.—*Female* (MCN 21994). Carapace dark yellow with gray pigment. Chelicerae and labium yellow; endites pale yellow, basally brown. Sternum yellow, with irregular light brown markings. Legs pale yellow with narrow dark bands; legs I and II darker ventrally; leg IV darker on the patella. Abdomen higher than longer. Dorsum brown with two wide paramedian white bands united anteriorly; a wide transversal white band limiting the anterior and the posterior area. Posterior area dark brown with a median-longitudinal white line that does not reach the transversal band. Abdomen laterally brown with white dots (Fig. 1l). Venter brown, with two circular white markings, one on eaeh side. Eyes red ringed. AME larger than the other eyes and separated from each other by almost a diameter. AME close to the ALE. PME apart from each other by little more than a diameter and separated from PLE by half of PME diameter. Measurements. Total length 1.70. Carapace: length 0.72, width 0.57. Abdomen: length 1.05, width 1.10, high 1.32. Legs, formula 1423, length I/II/III/IV: femora 0.85/0.62/0.44/0.67; patellae 0.32/0.27/0.22/0.30; tibiae 0.55/0.37/0.22/0.40; metatarsi 0.57/0.40/0.30/0.47; tarsi 0.42/0.35/0.30/0.35. Total legs length: 2.71/2.01/1.48/2.19.

Variation: Total length (n = 6) 1.62–1.87; carapace 0.67–0.77; femur I 0.85–0.92.

Male: unknown.

Distribution.—BRAZIL (*Amazonas*). (Fig. 3a)

Cryptachaea ericae **sp. nov.**
Figs. 2a–c, 3a

Type material.—*Holotype:* Female (MCN 9977), BRAZIL, Rio Grande do Sul, Espumoso, Salto do Jacuí, 14.i.1982, A.A. Lise leg.

Etymology.—The specific name honors the arachnologist Erica H. Buckup (MCN/FZBRS), in recognition for her many contributions to this manuscript. The species epithet is a genitive noun.

Diagnosis.—*Cryptachaea ericae* **new species** resembles *C. bonaldoi* Buckup, Marques and Rodrigues 2010 (see Buckup et al. 2010, Figs. 8, 9) by the epigynum with a well-demarcated depression, but differs from it by the transversal epigynal depression with projected posterior portion in the shape of a small scape (Fig. 2a, b).

Description.—*Female* (MCN 9977). Carapace yellow with a longitudinal band shaped as a "V," lateral borders and the ocular region dark brown. Chelicerae yellow with gray pigment. Labium and endites pale yellow. Sternum yellow with a dark brown border (Fig. 2c). Legs pale yellow, dark brown ringed; femora with many small dark stains anteriorly. Dorsum of the abdomen with dark brown pigment but without a well-defined color pattern; posterior median hump with a depigmented line until the spinnerets. Venter dark brown, except for a depigmented ring near the spinnerets. Eyes subequal. AME separated from each other by almost an eye's diameter and separated from the ALE by a radius of the AME. Posterior eyes equidistant, separated by almost the diameter of the PME. Measurements. Total length 5.85. Carapace: length 2.00, width 1.85. Abdomen: length, 3.85, width 3.84, high 4.65. Legs, formula 1423, length I/II/III/IV: femora 3.84/2.50/1.79/3.00; patellae 0.89/0.85/0.75/0.89; tibiae 2.35/1.40/0.95/1.75; metatarsi 3.25/1.95/1.35/2.30; tarsi 1.25/0.95/0.80/1.05. Total legs length: 11.58/7.65/5.64/8.99.

Male: unknown.

Distribution.—Brazil (*Rio Grande do Sul*) (Fig. 3a).

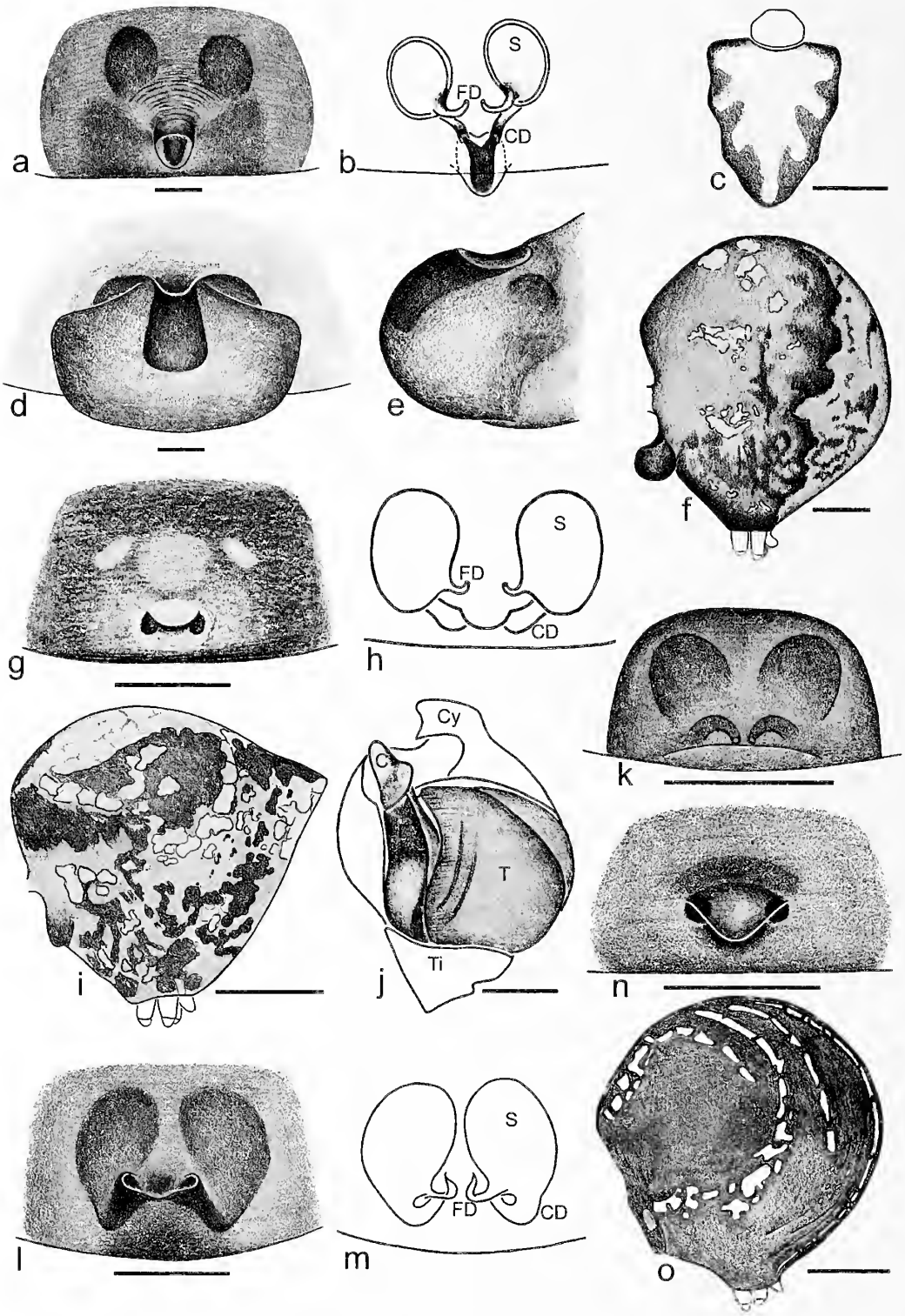

Figure 2a-o.—*Cryptachaea* **new species**. *Cryptachaea ericae* **new species**. a. epigynum, ventral; b. epigynum dorsal; c. female sternum. *Cryptachaea propinqua* **new species**. d. epigynum, ventral; e. epigynum, lateral; f. female habitus, lateral. *Cryptachaea catita* **new species**. g. epigynum, ventral; h. epigynum, lateral; i. female habitus, lateral. *Cryptachaea tambopata* **new species**. j. male palpus, ventral. *Cryptachaea paquisha* **new species**. k. epigynum, ventral. *Cryptachaea benivia* **new species.** l. epigynum, ventral; m. epigynum, dorsal. *Cryptachaea lavia* **new species**. n. epigynum, ventral; o. female habitus, lateral. Scale bars: a, b, d, e, g, h, j–n = 0.1 mm; o = 0.25 mm; c, f, i = 0.5 mm (C, conductor; CD, copulatory ducts; Cy, cymbium; E, embolus; FD, fertilization ducts; MA, median apophysis; S, spermathecae; T, tegulum; Ti, tibia).

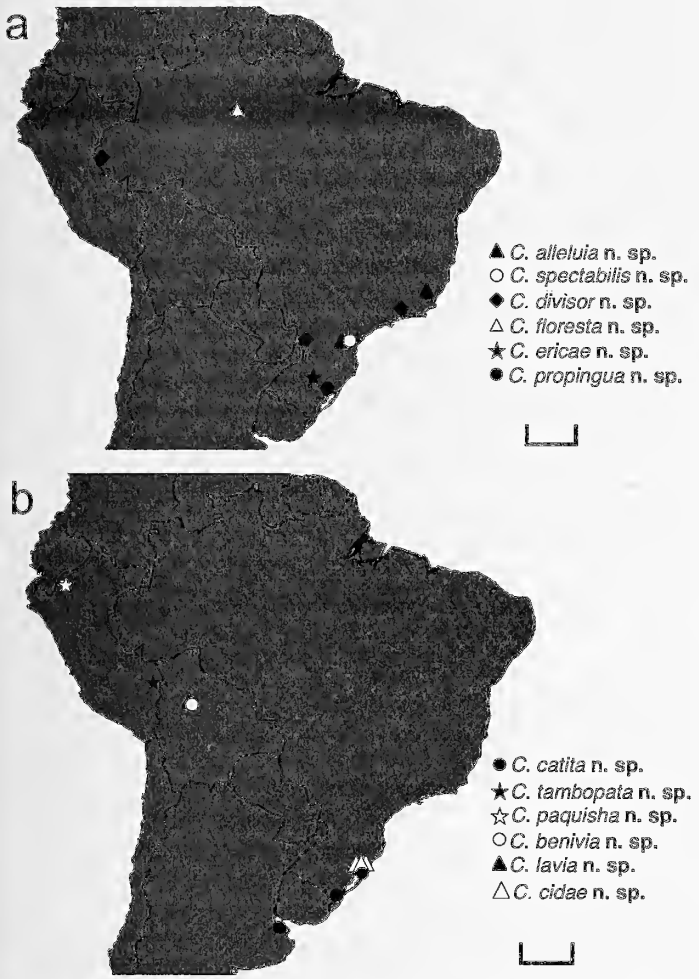

Figure 3a, b.—Distribution maps of *Cryptachaea* new species. Scale bars: 500 km.

Cryptachaea propinqua **new species**
Figs. 2d–f, 3a

Type material.—*Holotype:* Female (MCN 313), BRAZIL, *Rio Grande do Sul*, Guaíba, 03.i.1974, A.A. Lise leg.

Etymology.—The specific name is a Latin adjective that means "similar."

Diagnosis.—The female of *Cryptachaea propinqua* **new species** (Fig. 2e, f) resembles that of *C. gigantea* (Keyserling 1884) (see Levi 1963, Figs. 52–54) by the prominent and very sclerotized epigynum (Fig. 2e), but differs by the conspicuous longitudinal black stain in the epigynum close to the genital opening (Fig. 2d).

Description.—*Female* (MCN 313). Carapace dark brown (borders darker), with gray pigment and radial grooves. Chelicerae yellow, slightly gray. Labium and endites reddish-brown. Sternum reddish-brown (borders darker), except by a small central yellow marking. Legs pale yellow, ringed; coxae with dark brown apex. Abdomen higher than longer; dorsum yellow, with a group of white markings on each side (Fig. 2f); posterior area with white pigment, with a narrow dark brown band and a small irregular brown markings; laterally, on each side, two white stains. Venter yellowish-brown, laterally with two longitudinal paramedian white stains; spinnerets surrounded by dark brown pigment. AME smaller than the other eyes. AME separated from each other by about a half of an eye's diameter and placed close to the ALE. Posterior eyes with the same size and equidistance, separated from each other for about three-quarters of the PME. Measurements. Total length 3.34. Carapace: length 1.17, width 1.05. Abdomen: length 2.37, width 2.12, high 2.75. Legs, formula 1423, length I/II/III/IV: femora 1.87/1.22/0.87/1.50; patellae 0.57/0.47/0.37/0.52; tibiae 1.30/0.77/0.47/0.87; metatarsi 1.62/0.92/0.65/1.07; tarsi 0.75/0.55/0.47/0.57. Total legs length: 6.11/3.93/2.83/4.53.

Male: unknown.

Distribution.—BRAZIL (*Rio Grande do Sul*) (Fig. 3a).

Cryptachaea catita **new species**
Figs. 2g–i, 3b

Type material.—*Holotype:* Female (MCN 16162), BRAZIL, *Rio Grande do Sul*, Rio Grande, Estação Ecológica do Taim, 04.xii.1986, A.D. Brescovit leg. Paratypes: female (MCN 13995), Santa Vitória do Palmar, Estação Ecológica do Taim, 26.xi.1985, M.A.L. Marques leg.; female (MCTP 2208), Palmares do Sul, Praia do Quintão, 06.ix.1992, A.P. Petersen leg.; female (MLP 16283), ARGENTINA, *La Plata*, undated, R. Costa leg.

Etymology.—The specific name is a Latin adjetive that means "graceful."

Diagnosis.—The epigynum of *Cryptachaea catita* **new species** (Fig. 2g, h) resembles that of *C. passiva* (Keyserling 1891) (see Levi 1963, Figs. 26, 27) by the shape of the copulatory openings, but differs by the conspicuous median hump at the apex of the abdomen, which is absent in *C. catita* (Fig. 2i).

Description.—*Female* (Holotype). Carapace dark brown, with dark grooves and yellow stains and wide pale yellow bands extending laterally in the thoracic region. Labium and endites pale yellow. Chelicerae yellowish-gray. Sternum pale yellow, with light brown stains running laterally, and three small humps on each side. Legs pale yellow; all femora and tibiae anteriorly with dark bands; distal portion of the metatarsi ringed. Abdomen with a dorsal median hump. Dorsum with white and black pigment; lateral area black with yellow stains. Venter with black and yellow stains, and two paramedian white bands. Subequal eyes. AME separated from each other by about an eye's diameter and separated from the ALE by one-fifth of the AME diameter. Posterior eyes equidistant, separated from each other by about three-fourths of the PME. Measurements. Total length 2.47. Carapace: length 0.80, width 0.70. Abdomen: length, 1.60, width 1.17, high 1.52. Legs, formula 1423, length I/II/III/IV: femora 1.22/0.80/0.62/1.00; patellae 0.37/0.32/0.27/0.35; tibiae 0.75/0.50/0.35/0.57; metatarsi 0.85/0.55/0.40/0.62; tarsi 0.47/0.35/0.32/0.40. Total legs length: 3.66/2.52/1.96/2.94.

Variation: (n = 4) Carapace length, 0.80–0.97; femur I, 1.22–1.47. Sternum may have a brown border.

Male: unknown.

Distribution.—BRAZIL (*Rio Grande do Sul*) and ARGENTINA (*La Plata*) (Fig. 3b).

Cryptachaea tambopata **new species**
Figs. 2j, 3b

Type material.—*Holotype:* male (MUSM), PERU, *Madre de Dios*, Zona Reservada Tambopata, 23.vii.1987, D. Silva leg.

Etymology.—The specific name is a noun in apposition taken from the type locality.

Diagnosis.—The male palpus of *Cryptachaea tambopata* **new species** resembles to those of *C. pura* (O. P.-Cambridge 1894) (see Levi 1959, Fig. 44), but differs from that species by the apex of the cymbium with one obtused tip and one acuted tip. The conductor is small with an obtuse apex (Fig. 2j).

Description.—*Male* (Holotype). Carapace yellowish-gray, darker in the median-longitudinal area. Chelicerae with gray pigment and two teeth in the promargin. Labium (fused to the sternum), endites and sternum whitish; sternum with brown borders. Legs whitish with dark bands in the middle of the distal portion of the segments; anterior femora ventrally gray. Abdomen dorsum yellow, black stained, with yellow areas and some white pigment; posterior area with a median-longitudinal white line. Venter darker, with two small white stains, one on each side, between the epigastric furrow and the spinnerets. Subequal eyes. AME separated from each other by a half an eye's diameter and close to the ALE; PME separated from each other by a radius and from the PLE by one-quarter of a diameter. Measurements. Total length 1.60. Carapace: length 0.75, width 0.67. Abdomen: length 0.82, width 0.70, high 0.95. Legs, formula 1423, length I/ II/III/IV: femora 1.05/0.67/0.47/0.77; patellae 0.32/0.27/0.22/0.27; tibiae 0.65/0.42/0.30/0.45; metatarsi 0.67/0.45/0.32/0.50; tarsi 0.50/0.37/ 0.27/0.37. Total legs length: 3.19/2.18/1.58/2.36.

Female: unknown.

Distribution.—PERU (*Madre de Dios*) (Fig. 3b).

Cryptachaea paquisha **new species**
Figs. 2k, 3b

Type material.—*Holotype:* female (MUSM), PERU, *Amazonas*, alto río Comaina "Falso Paquisha," Puesto de vigilancia 22, 850–1150 m, 21.x–3.xi.1987, D. Silva leg.

Etymology.—The speeific name is a noun in apposition taken from the type locality.

Diagnosis.—Female of *Cryptachaea paquisha* **new species** differs from the other species by the epigynum protruding moderately with curved ducts, visible by transparency (Fig. 2k).

Description.—*Female* (Holotype). Carapace, labium, endites, chelicerae, and sternum yellow with gray pigment. Legs dark yellow, except femora I pale yellow with distal part gray, ventrally; other segments with dark bands. Abdomen dorsum yellow, with some areas light brown; two white stains on each side, and brown between. Venter dark, with two paramedian white stains between the epigastric furrow and spinnerets. Spinnerets surrounded by a black ring. AME bigger than the other eyes, separated from each other by an eye's radius and close to the ALE. PME separated from each other by an eye's diameter and separated from the PLE by two-thirds of the PME diameter. Measurements. Total length 2.07. Carapace: length 0.85, width 0.72. Abdomen: length 1.20, width 1.05, high 1.30. Legs, formula 1423, length I/II/III/IV: femora 1.07/0.75/0.52/0.90; patellae 0.40/0.35/0.27/0.35; tibiae 0.67/0.42/0.25/0.52; metatarsi 0.72/0.47/0.32/0.55; tarsi 0.47/0.40/ 0.32/0.42. Total legs length: 3.33/2.39/1.68/2.74.

Male: unknown.

Distribution.—PERU (*Amazonas*) (Fig. 3b).

Cryptachaea benivia **new species**
Figs. 2l, m, 3b

Type material.—*Holotype:* Female (USNM), BOLIVIA, *Beni*, Estación Biologica del Beni, 08–14.ix.1987, S. Larcher & J. Coddington leg.

Etymology.—The specific name is a eombination of letters from the type locality and Bolivia.

Diagnosis.—The epigynum of *Cryptachaea benivia* **new species** (Fig. 2l, m) resembles that of *C. banosensis* (Levi 1963) (see Levi 1963, Figs. 60, 61) by the shape of the depression of the epigynum, but differs from it by the internal duct trajectory (Fig. 2m), and by the lack of the large black dots.

Description.—*Female* (Holotype). Carapace dark yellow, gray pigmented. Labium and endites yellowish-gray. Sternum dark brown. Coxae pale yellow, except coxae I gray. Legs pale yellow; femora ventrally with the distal portion dark yellow; patellae and other segments gray dorsally; distal portion of the tibiae and the base of the metatarsi dark gray; tibiae IV with distal dark bands. Abdomen, longer than higher; dorsum gray with a median-longitudinal white band, anteriorly, followed by two pairs of paramedians white stains, surrounded a darker median area in the middle; two transversal white lines, one on each side; white spots near to the spinnerets. Venter black with three median white marks. Subequal eyes. AME largest eyes, separated from each other by almost three-quarters of eye diameter and close to the ALE. Posterior eyes equidistant, separated from each other by the PME diameter. Measurements. Total length 3.05. Carapace: length 1.42, width 1.15. Abdomen: length 2.00, width 1.85, high 1.57. Legs, formula 1423, length I/ II/III/IV: femora 2.25/1.37/1.00/1.82; patellae 0.67/0.52/0.47/0.62; tibiae 1.57/0.77/0.55/1.00; metatarsi 2.00/ 1.17/0.80/1.42; tarsi 0.90/0.65/0.52/0.70. Total leg length: 7.39/ 4.48/3.34/5.56.

Male: unknown.

Distribution.—BOLIVIA (*Beni*) (Fig. 3b).

Cryptachaea lavia **new species**
Figs. 2n, o, 3b

Type material.—*Holotype:* Female (MCN 24687), BOLIVIA, *Beni*, Estaeión Biologica del Beni, 27–29.vii.1993, H. Höfer & A.D. Brescovit leg.

Etymology.—The specific name is a random eombination of letters.

Diagnosis.—The epigynum of *Cryptachaea lavia* **new species** (Fig. 2n) resembles that of *C. manzanillo* (Levi 1959) (see Levi 1959, Figs. 27, 28) by the median lobe in the anterior border of the epigynum, but differs from it by the color pattern (Fig. 2o) and by the closely positioned copulatory openings (Fig. 2n).

Description.—*Female* (MCN 24687). Carapace, labium, endites, and sternum dark yellow with brown pigment. Chelicerae yellow, promargin with one tooth. Legs dark yellow, except by the proximal and distal portion of the femora, which is pale yellow; coxae pale yellow, gray stained. Abdomen yellow; dorsum, anteriorly, with two paramedian white bands and then, three narrow white lines on each side, descendant laterally, but only the median one reaehes the pulmonary area; posterior area with a narrow median-longitudinal white line, from the spinnerets to the third lateral line (Fig. 2o). Eyes subequal. AME separated from each other by two-third of its diameter and close to the ALE. Posterior eyes equidistant, separated from each other by two-thirds of the PME diameter. Measurements. Total length 2.42. Carapace: length 0.72, width 0.65. Abdomen: length 0.72, width 1.47, high 1.75. Legs, formula 1423, length I/II/III/IV: femora 0.97/0.72/0.55/0.80; patellae 0.32/0.27/0.20/0.32; tibiae 0.62/

0.37/0.30/0.72; metatarsi 0.70/0.47/0.37/0.55; tarsi 0.44/0.37/0.30/0.40. Total legs length: 3.05/2.20/1.72/2.79.

Male: unknown.

Distribution.—BOLIVIA (*Beni*) (Fig. 3b).

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Natural history and two new species of the trapdoor spider genus *Conothele* Thorell 1878 (Araneae: Ctenizidae) from India

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Abstract. Two new species of the genus *Conothele* Thorell 1878 of the trapdoor spider family Ctenizidae are described from India: *Conothele giganticus,* sp. nov. is described from the Ngengpui Wildlife Sanctuary, Mizoram in northeast India and *C. khunthokhanbi* sp. nov. is described from Manipur in northeast India. Like other known Indian *Conothele* species, these spiders are also strictly ground burrowing. Additional information on habitat and natural history is provided for both species.

Keywords: Manipur, Mizoram, Mygalomorphae

The genus *Conothele* Thorell 1878 currently contains 18 species over the Oriental and Australian regions (Decae 2010; Platnick 2014). It is closely related to the genus *Ummidia* Thorell 1875 (Hedin & Bond 2006, Bond et al. 2012) from which it differs only in characters of undetermined generic significance (Main 1997; Siliwal et al. 2009; Opatova et al. 2013). With better sampling across their distribution ranges and the incorporation of molecular tools, we will better understand the validity of the two genera (Opatova et al. 2013). The different species of *Conothele* vary in body size, burrowing habit (arboreal or ground burrowing), and behavior (modifications in burrow structure) (Pocock 1900; Gravely 1935; Siliwal et al. 2009). This could be an adaptation to the local habitat.

So far, only two Indian *Conothele* species have been described (Siliwal et al. 2009). Here, we report on the discovery of two additional new species from Mizoram and Manipur, based on female specimens, and provide basic natural history information for the species from Mizoram. In this group of spiders, females have shown a distinct spermathecae structure with no variation that proves to be a useful character in species identification in the absence of the male. In contrast to previous reports of them being arboreal (Pocock 1900; Gravely 1935), all known Indian species of *Conothele* are ground burrowing.

METHODS

The specimens are deposited at the museum collection of the Wildlife Information Liaison Development Society, Coimbatore, Tamil Nadu. Measurements of body parts except for eyes were taken with a MitutoyoTM Vernier Caliper. Eye measurements were made with a calibrated ocular micrometer. All measurements are in mm and are accurate to ± 0.02. Spermathecae were dissected and cleared in concentrated lactic acid. All illustrations were prepared with the help of a camera lucida attached to a MOTIC^{TM} stereomicroscope by MS.

Abbreviations: ALE = anterior lateral eye, AME = anterior median eye, MOA = median ocular area, PLE = posterior lateral eye, PLS = posterior lateral spinnerets, PME = posterior median eye, PMS = posterior median spinnerets, PER = posterior eye row, WILD = Wildlife Information Liaison Development Society. Abbreviations used for hair and spines count are d = dorsal, fe = femur, mt = metatarsus, p = prolateral, pa = patella, r = retrolateral, ta = tarsus, ti = tibia, v = ventral.

TAXONOMY

Family Ctenizidae Thorell 1887
Genus *Conothele* Thorell 1878

Remarks.—As with other ctenizids, the females have three claws and lack scopula and claw tufts.

KEY TO FEMALES OF *CONOTHELE* SPECIES OF INDIA

1. Eye group about as wide posteriorly as anteriorly and at least twice as wide as long 2
   Eye group clearly narrower posteriorly and only about 1.5 times wider than long ... *C. varvarti* Siliwal, Nair, Molur and Raven
2. Females with carapace length of 5–6mm and with spines distally on prolateral tibia III either weak or absent 3
   Females with carapace length of 10–13mm and strong spines distally on prolateral tibia III (Fig. 1F) ..... *C. giganticus* **sp. nov.**
3. Eye group twice as wide as long and cuspules on maxillae in one group ..... *C. vali* Siliwal, Nair, Molur and Raven
   Eye group clearly more than twice as wide as long and cuspules on maxillae in two groups ..... *C. khunthokhanbi* **sp. nov.**

***Conothele giganticus* Siliwal and Raven** new species

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(Figs. 1A–H, 2A–C)

Type specimens.—INDIA: Mizoram: Holotype female, Ngengpui Wildlife Sanctuary, Mizoram, elev. 217 m, 22.51691° N, 92.77319° E, 12 October 2012, Manju Siliwal (WILD-12-ARA-1162); paratypes 2 females, same data as holotype, 13 March 2013, Manju Siliwal and Keshab Gogoi (WILD-13-ARA-1207, 1208).

Diagnosis.—*Conothele giganticus* new species differs from other known species of this genus from India and Myanmar by the very large body, almost double the size of other known

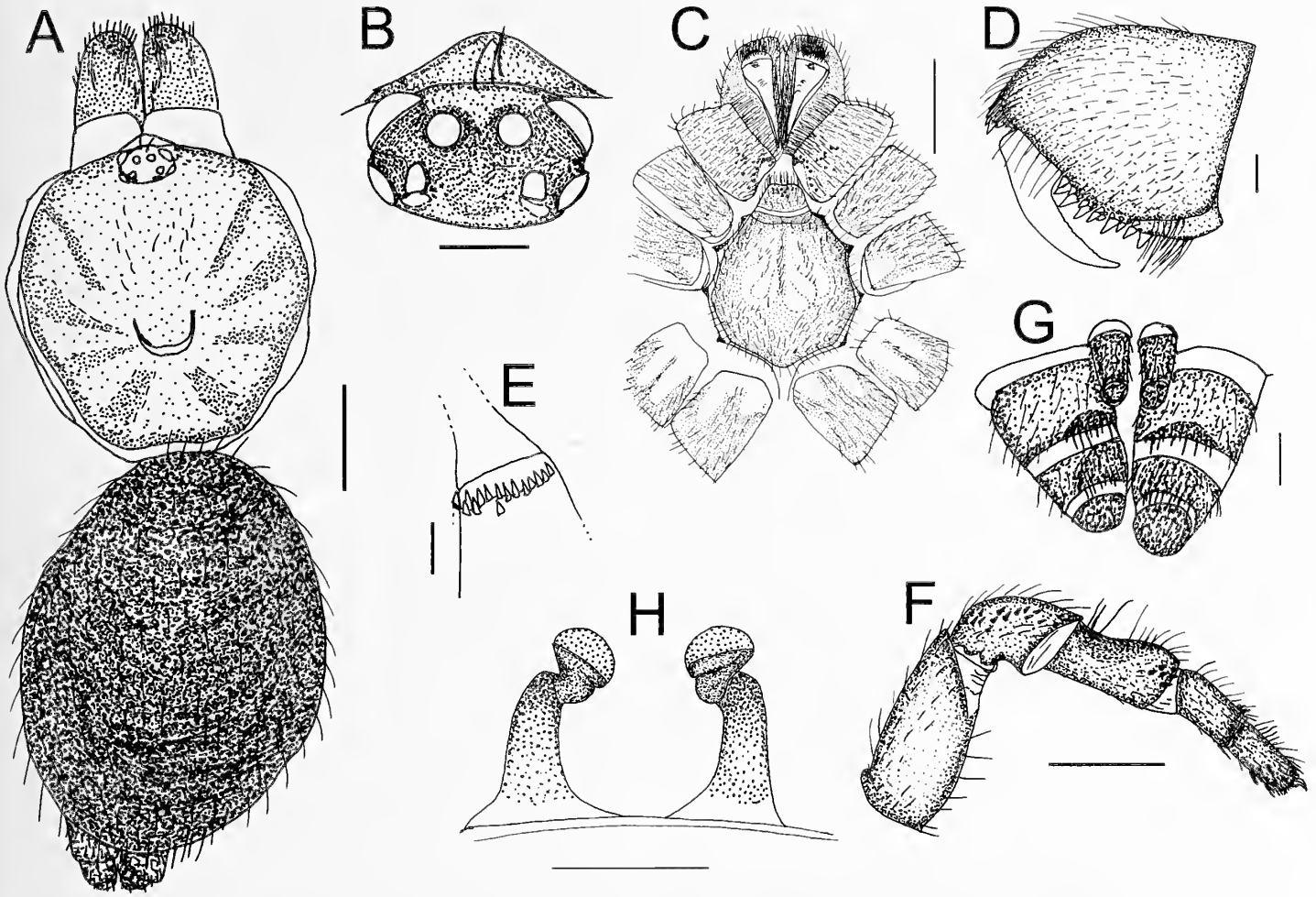

Figure 1.—*Conothele giganticus* new species, female (WILD-12-ARA-1162). A, Carapace and abdomen, dorsal view; B, Eyes; C, Sternum, maxillae, labium, chelicerae; D, Chelicerae, prolateral view; E, Chelicerae, rastellum spines. lateral view; F, Leg III (Fe to Ta) prolateral view, I; G, Spinnerets, ventral view; H, Spermathecae. Scale 1.0 mm for B, D, E, G, H and scale 5.0 mm for A, C, F.

species of Indian *Conothele*; carapace distinctly longer than patella and tibia of leg I and IV (in *C. vali* Siliwal, Nair, Molur & Raven 2009, carapace shorter than patella plus tibia of leg I and IV); by the anterior and posterior eye groups about equally wide (eye group clearly narrower posterior in *C. varvarti* Siliwal, Nair, Molur & Raven 2009) and together at least twice as wide as long (about 1.5 times in *C. varvarti* and *C. vali*; more than twice in *C. khunthokhanbi* new species); strongly spined on distal prolateral face of tibia III (absent in *C. khunthokhanbi* new species, 2–3 spines in *C. varvarti* and *C. vali*); spermathecae with globular apical lobe facing up, each stalk broader at the base, distally gradually narrowing down and at the base of lobe stalk is sclerotized and partially bent in zigzag pattern.

Etymology.—The species epithet is derived from Latin word for "large," referring to the putatively diagnostic larger size of spider compared to other species of *Conothele* described from India and Myanmar.

Description Holotype female.—Total length, 31.32; carapace 13.42 long, 12.99 wide. Abdomen 17.90 long, 13.14 wide. Spinnerets: PMS, 1.18 long, 0.85 wide, 0.16 apart; PLS, 2.99 total length (1.74 basal, 0.86 middle, 0.39 distal; midwidths 2.45, 1.91, 1.33 respectively). Morphometry of legs and palp are given in Table 1.

Color in life: Complete spider black (Fig. 2C).

Color in alcohol: Carapace reddish-brown, darker near fovea, striae and margins. Sternum orangish-brown, darker anteriorly and on margins. Labium, coxae, maxillae reddish-brown. Chelicerae blackish-brown dorsally. Abdomen grayish-black, dorsally with scattered small yellowish spots radiating in curved line; ventrally, integument appears wrinkled with paler or yellowish furrows.

Carapace: Glabrous except for 17 long bristles on caput, few short on thoracic region along striae, three between anterior eyes (Fig. 1A), weak crenulations on caput, more conspicuous near eye, caput. Caput with distinct mound between fovea and eyes. Fovea deep, procurved, U-shaped (Fig. 1A).

Eyes (Fig. 1B)*:* Eight in two rows on distinct ocular tubercle, both rows procurved, posterior row slightly procurved, ocular group 1.26 long, 2.52 wide, ~0.28 of head width; MOA, 1.40 anterior width, 1.62 posterior width, 1.05 long. AME 0.41, PME 0.36, ALE 0.69, PLE 0.43; distance between ALE-AME 0.16, AME-AME 0.16, PLE-PME adjacent, PME-PME 1.08, ALE-PLE 0.08; clypeus, very narrow or absent, chilum distinct, triangular.

Maxillae (Fig. 1C)*:* 3.94 long anterior, 5.68 long posterior, 3.24 wide; 25 cuspules 2–3 irregular short rows on prolateral-

Table 1.—Morphometry of legs and palp of the holotype female (WILD-12-ARA-1162) and paratype female (WILD-13-ARA-1208) of *Conothele giganticus* new species.

	Leg I		Leg II		Leg III		Leg IV		Palp	
	Holo	Para	Holo	Para	Holo	Para	Holo	Para	Holo	Para
	#1162	#1208	#1162	#1208	#1162	#1208	#1162	#1208	#1162	#1208
					Lengths (mm)					
Femur	8.48	6.57	7.14	5.23	7.25	5.64	9.43	7.76	8.58	6.92
Patella	5.99	4.36	5.38	3.66	5.46	3.61	5.58	3.68	5.2	3.49
Tibia	5.55	4.37	4.2	2.96	4.75	2.76	5.78	4.56	5.67	4.57
Metatarsus	3.71	2.55	3.4	2.01	3.56	2.26	5.86	4.09	-	-
Tarsus	2.39	1.37	2.49	1.44	3.59	2.55	3.84	2.02	4.47	3.29
Total	26.12	19.22	22.61	15.3	24.61	16.82	30.49	22.11	23.92	18.27
					Midwidths (mm)					
Femur	2.38	2.24	2.33	2.24	2.58	2.51	2.52	2.17	2.01	2.26
Tibia	2.55	1.47	2.47	1.57	3.34	1.86	2.84	1.72	2.38	1.27

proximal corner, half length of maxillae. Anterior lobe greatly reduced.

Labium (Fig. 1C)*:* 2.03 long, 2.66 wide, labiosternal groove shallow, straight with procurved ends, 10 large cuspules in two rows (6+4) centrally, size of cuspules similar to that on maxillae.

Chelicerae (Figs. 1D, E)*:* 6.95 long; 11 large promarginal teeth, 9 large and 1 very small retromarginal teeth, basomesal teeth absent; rastellum conspicuous, raised on low mound, consist of 14 thick spines on vertical face and up, 10 of which make up anterior row; many long and short bristles present along anterior dorsal surface.

Sternum (Fig. 1C)*:* broader between coxae III–IV, orangish-brown, elevated towards margins, sloping posteriorly, 7.60 long, 7.46 wide, covered with long black bristles, more dense towards lateral sides, posterior angle blunt and not separating coxae IV. Sigilla large, irregularly shaped centrally placed. Non-sigillate area with fine corrugations.

Legs: III–IV thicker than I–II. Tibiae, metatarsi, and tarsi of I–II and tibiae and tarsi of palp dorsoventrally flattened. Femora III clearly wider than the rest. Tibiae III with saddle-shape depression on lower basal half (Fig. 1F). Metatarsi III shorter than tarsi III. Legs covered with sparsely distributed

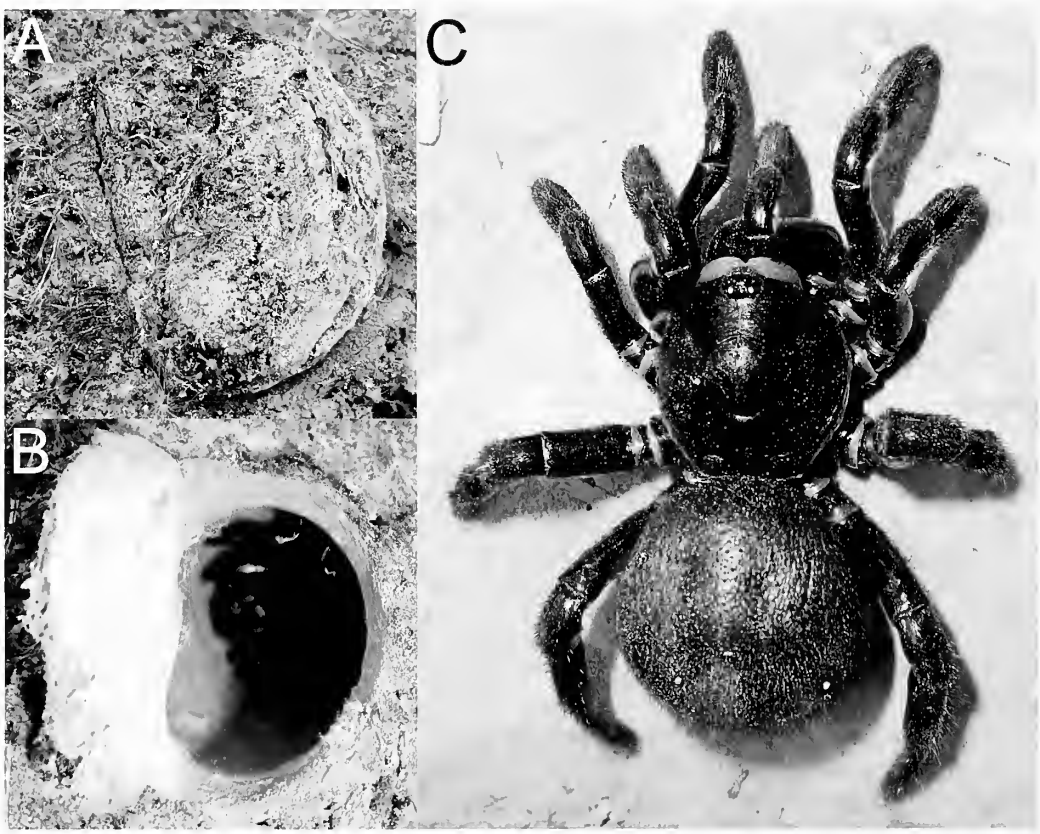

Figure 2.—*Conothele giganticus* new species, female (WILD-12-ARA-1162). A, Burrow closed; B, Burrow open; C. spider in life.

hair, bristles, curved and normal thick thorn-like spines. Two conspicuous hairless bands running over length of femora, patellae, and tibiae. Apophysis on dorsal trochanter III present. Leg formula 4132.

Spines: curved thick thorn-like spines on legs I–II and palp, rest normal spines. I: ti p = 76, r = 70, mt p = 66, r = 42, ta p = 29, r = 13; II: ti p = 50, r = 26, mt p = 46, r = 13, ta p = 30, r = 9; III: pa p = 14, ti p = 5, mt p = v = 4, r = 3, ta p = 18, r = 5; IV: mt p = 4, ta p = 19, r = 4; palp: pa v = 1, ti p = 82, r = 74, ta p = 70, r = 62.

Trichobothria: ti I–IV, 14–20 in four rows on lateral side amongst spines; mt I–IV with 16–26 filiform trichobothria in four rows for length; ta I with 3 clavate trichobothria basal, 20–24 filiform in multiple longitudinal rows; ta II with 6 clavate in basal, 20–24 filiform in two longitudinal rows; ta III with 3 clavate in basal one fourth, 11 filiform in multiple longitudinal rows; ta IV with 4 clavate basal, 9 filiform in two rows in distal half; palp, ti with 6 (rest broken) filiform in two curved rows; ta with 6 clavate in center, 8 filiform in 2 longitudinal rows.

Leg coxae: coxae IV clearly wider than rest, I clearly longer than rest, II and III subequal. Covered with black bristle-like hair; hairless or glabrous bands more prominent on III–IV; small spot on basal area of I–II, distinct two glabrous bands for length on III, three glabrous bands in basal two third on IV.

Claws: paired claws on legs III–IV with unequal bifid tooth and slightly larger than on I–II each with one tooth. Palpal claw with one tooth.

Abdomen: grayish-brown, with few small yellow spots radiating in curved line, covered with short and long black bristles on tubercles, giving warty appearance; one in 7–8 bristle is long and about 2–3 times longer and slightly thicker than short bristles. Ventrally, uniformly covered with short and long bristles.

Spinnerets: PMS digitiform covered with black hair; PLS, covered with black hair, apical segment dome-shape (Fig. 1G), ventrally with band of spigots (two types), basal segment with short band on distal edge, middle segment with long band for length, apical segment with hairs only on periphery, rest covered with spigots.

Spermathecae (Fig. 1H)*:* paired lobes, each stalk broader at base, gradually narrowing distally with globular apical swollen lobe, upwards-facing stalk is sclerotized and partially bent in zigzag pattern at base of lobe .

Paratype female (WILD-13-ARA-1208).—Total length 23.70; carapace 10.78 long 9.70 wide; chelicerae 4.85 long intact, 9 large and 3 very small retromarginal and 9 large and 2 small promarginal teeth. Sternum 5.24 long, 5.10 wide. Labium 1.55 long, 1.35 wide, 11 large cuspules. Maxillae 2.45 long anteriorly, 4.29 long posteriorly, 1.97 wide, 22 cuspules. Abdomen 12.92 long and 9.29 wide. Spinnerets: PMS, 0.95 long, 0.46 wide, 0.15 apart; PLS, 1.04 total length (1.04 basal, 0.35 middle, 0.1 distal; midwidths 1.44, 1.29, 0.41 respectively). Spines: I: pa v = 1; ti p = 58, r = 63, mt p = 49, r = 38, ta p = 28, r = 15; II: ti p = 39, r = 17, mt p = 39, r = 8, ta p = 26, r = 7; III: pa p = 13, ti p = 5, mt d = 6, p = 5; ta p = 17, r = 3; IV: ti v = 2 (only on one leg), mt p = 6, ta p = 18, r = 4; palp: pa v = 1–2, ti p = 66, r = 69, ta p = 52, r = 53. Morphometry of leg and palp is given in Table 1. Rest of the characters are same as holotype (WILD-12-ARA-1162).

Distribution.—India, Mizroam: Ngengpui, Mampui in Lawngtlai district.

NATURAL HISTORY

This species (Fig. 2C) was found alongside road cuts in Mampui and Ngengpui Wildlife Sanctuary and we presume it occurs in all the forested area surrounding these two localities. Burrows were difficult to locate as they were highly camouflaged and in dense undergrowth. Burrows were relatively easier to find during the dry season than in the wet season. We could locate only eight burrows, of which only three were inhabited. Abandoned burrows had the trapdoor lid open and were hinged at the side. Burrows were simple, short (55–80 mm), tube-like, wider at the base and with D-shape trapdoor lid closing the entrance (Fig. 2A). The trapdoor lid was thick and cork-like, as seen in other members of this family, the door hinge was always on the sides of the entrance but none were seen at the top or bottom (Fig. 2B). The outer side of the lid was covered with soil, moss, and debris while the inner side of the lid had silk knitted parallel to the lid hinge and fang marks were evident. During the dry season, an additional rim of silk was attached to the periphery of the lid as well as to the burrow entrance to ensure a tighter fit, presumably to help better maintain optimum temperature and humidity inside the burrow. Gravid females were collected during March, which indicated that these spiders nest during summer.

Burrows were found on road-side cuts on forest paths from 0.25 m to 2.5 m height, perpendicular to the angle of the slope of roadside cuts. They were close to the ground and higher up to 1.5 m, both in open and closed canopy areas. Burrows ranged between 20 × 15 mm to 35 × 27 mm diameter about 55–80 mm deep with lid thickness ranging from 4–6 mm. The habitat was evergreen forest in a wide elevation range from 100–800 m and the spider seemed to prefer areas with moderate to high canopy cover (< 50%) in a non-rocky area. We were able to locate them only in areas with less ground vegetation (2–15%).

Conothele khunthokhanbi Kananbala, Bhubaneshwari and Siliwal new species

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(Figs. 3A–F)

Type specimen.—INDIA: Manipur: holotype female, Kwakeithel, District Imphal, 14 October 2010, Akham Kananbala and M. Bhubaneshwari (WILD-10-ARA-542).

Diagnosis.—*Conothele khunthokhanbi* new species differs from other known *Conothele* species by the eye group with eyes set about as wide posteriorly as anteriorly (eye group clearly narrower posteriorly in *C. varvarti*) and more than twice as wide as long (about 1.5 times in *C. varvarti* and *C. vali*; twice in *C. giganticus* new species); spines absent on tibia III (spines present in *C. giganticus* new species, *C. varvarti* and *C. vali*); carapace distinctly longer than patella and tibia of leg I and IV as in *C. giganticus* new species (in *C. vali*, carapace shorter than patella and tibia of leg I and IV); cuspules on maxillae in two groups, one group in posterior basal half and second group in anterior lobe (one group in prolateral-proximal corner covering three fourths of maxillae length in *C. vali*, *C. varvarti*); spermathecae with globular apical lobe facing up, each stalk straight without any bend (Fig. 3F).

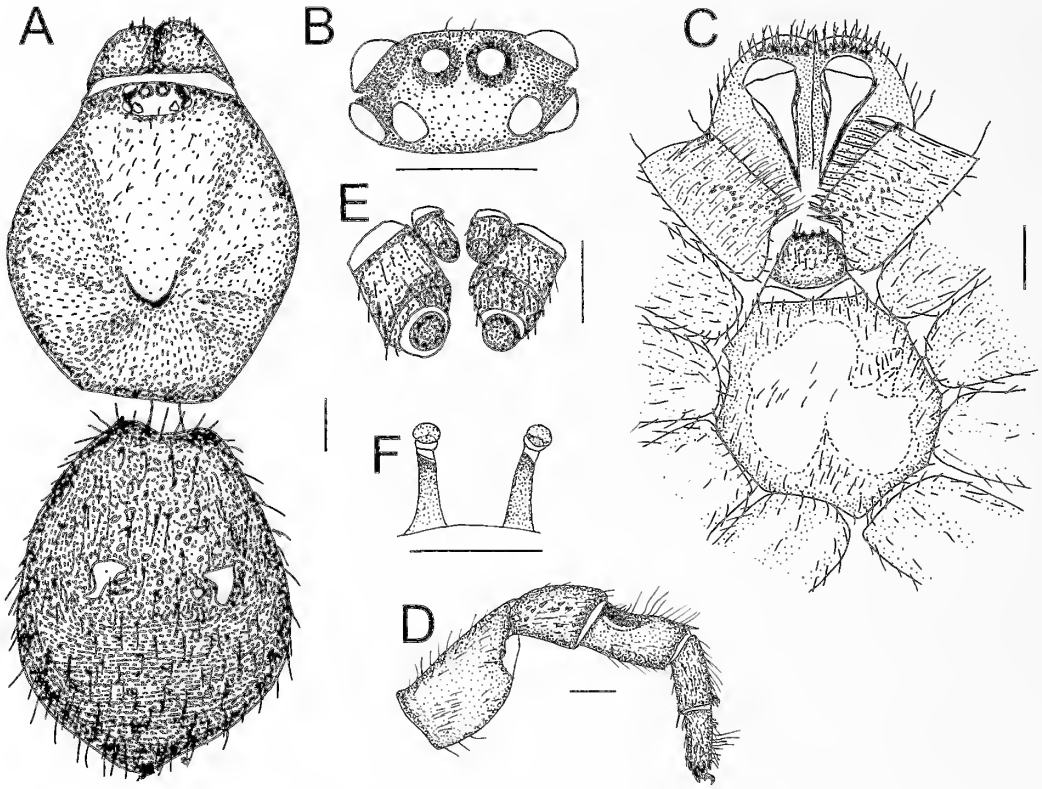

Figure 3.—*Conothele khunthokhanbi* new species, female (WILD-12-ARA-1162). A, Carapace and abdomen, dorsal view; B, Eyes; C, Sternum, maxillae, labium, chelicerae; D, Leg III (Ti to Ta) prolateral view, I; E, Spinnerets, ventral view; F, Spermathecae. Scale 1.0 mm for A–F.

Etymology.—The species epithet is a noun in apposition for the local goddess Khunthokhanbi in Manipur.

Description holotype *female*.—Total length, 14.26; carapace 6.74 long, 6.07 wide. Abdomen 7.52 long, 5.76 wide. Spinnerets: PMS, 0.63 long, 0.30 wide, 0.21 apart; PLS, 2.99 total length (0.70 basal, 0.18 middle, 0.10 distal; midwidths 0.97, 0.62, 0.50 respectively). Morphometry of legs and palp is given in Table 2.

Color in alcohol: Carapace, legs, chelicerae, reddish-brown, posterior legs lighter than anterior legs. Sternum yellowish-brown, labium, coxae, maxillae greenish-brown; abdomen, grayish-brown; dorsally with scattered small pale spots radiating in curved line; ventrally, integument yellowish with few black patches.

Carapace: Glabrous with few bristles on caput and ocular area. Bristles: *ca.* 50 small on caput and 2 small on clypeus edge. Weak crenulations on caput, more conspicuous near eyes, and caput. Caput with distinct mound between fovea and eyes. Fovea deep, procurved, U-shaped (Fig. 3A).

Eyes (Fig. 3B)*:* Eight in two rows on low ocular tubercle, both rows straight, clypeus absent. Ocular group 0.60 long, 1.42 wide; MOA 0.70 anterior width, 0.97 posterior width, 0.56 long. AME 0.21, PME 0.19, ALE 0.29, PLE 0.26; distance between ALE-AME 0.14, AME-AME 0.09, PME-PME 0.51, ALE-PLE and PLE-PME adjacent; clypeus absent or very narrow, chilum distinct, triangular.

Maxillae (Fig. 3C)*:* 1.86 long in anterior, 2.31 long posterior, 1.27 wide; cuspules in two groups, first group of 24–25 cuspules 2–3 evenly spread curved rows on prolateral-proximal corner for two-thirds length of basal maxillae and another small group of 7–8 cuspules on anterior-distal corner (anterior lobe area). Anterior lobe indistinct.

Labium (Fig. 3C)*:* 0.90 long, 1.20 wide, labiosternal groove shallow, straight with procurved ends with two labiosternal sigilla on either end of groove, 9 large cuspules in two curved rows (5+4) centrally, size of cuspules similar to that on maxillae.

Chelicerae (Fig. 3C)*:* 3.24 long; 4 large and one small promarginal teeth, 8 large retromarginal teeth, basomesal teeth absent; rastellum conspicuous, raised on low mound, consists of 25 thick spines on vertical face and up, of which, 11 in anterior row; many long and short spines along anterior dorsal surface.

Sternum (Fig. 3C)*:* 3.69 long, 3.36 wide, elevated towards margins, sloping posteriorly, broader between coxae III;

Table 2.—Morphometry of legs and palp of holotype female (WILD-10-ARA-542) of *Conothele khunthokhanbi* new species.

	Leg I	Leg II	Leg III	Leg IV	Palp
			Lengths (mm)		
Femur	3.96	3.5	3.29	4.23	3.87
Patella	2.58	2.55	2.22	2.85	2.36
Tibia	2.5	2.16	2.1	2.41	2.24
Metatarsus	1.96	1.41	1.15	2.7	-
Tarsus	1.15	1.42	1.44	1.77	2.38
Total	12.15	11.04	10.2	13.96	10.85
			Midwidths (mm)		
Femur	1.23	1.1	1.66	1.1	0.85
Tibia	1.27	1.02	1.23	1.16	1.19

covered with long black bristles, row of long bristles on margins, posterior angle blunt and not separating coxae IV. Sigilla large, irregular shape, centrally placed, covering most of sternum. Non-sigillate area with fine corrugations.

Legs: III–IV thicker than I–II. Tibiae, metatarsi and tarsi of I–II and tibiae and tarsi of palp dorsoventrally flattened. Femora III clearly wider than the rest. Tibiae III with saddle-shaped depression on lower basal half (Fig. 3D). Metatarsi III shorter than tarsi III. Legs covered with sparsely distributed hair, bristles, normal and curved thick thorn-like spines. Two conspicuous hairless bands for length of femora, patellae and tibiae. Apophysis on dorsal trochanter III present. Leg formula 4123.

Spines: curved thick thorn-like spines on legs I–II and palp, rest normal spines. I: ti p = 60, r = 65, mt p = 44, r = 34, ta p = 31, r = 26; II: ti p = 45, r = 11, mt p = 44, r = 13, ta p = 43, r = 13; III: pa p = 11, mt d = 4, v = 1, r = 5, ta p = 16, r = 3; IV: mt p = 3, d = r = v = 1, ta p = 13, r = 4; palp: pa p = 2, ti p = 54, r = 55, ta p = 52, r = 56.

Trichobothria: Ta I 4 clavate, 8 filiform for length; ta II 4 clavate, 8 filiform for length; ta III 6 clavate, 10 filiform in distal two third; ta IV, 7 clavate, 12 filiform in two rows in distal three quarters; palp, ta 9 clavate in center, 4 filiform in distal two thirds. Clavate only in basal area on all tarsi.

Leg coxae: Coxae IV clearly wider than rest, I clearly longer than rest, II and III subequal. Covered with black bristle-like hairs; hairless or glabrous bands more prominent on III–IV; small spot on basal area of II, distinct two glabrous bands for length on III, three glabrous bands in basal two thirds on IV.

Claws: paired claws on legs III–IV with unequal bifid tooth and slightly bigger than on I–II, each with single tooth. Palpal claw with single bifid tooth.

Abdomen: grayish-brown, with few yellow small spots radiating in curved line, covered with short and long black bristles on tubercles, giving warty appearance; one in each 6–7 bristles is long and about 2–3 times longer and slightly thicker than short bristles. Ventrally, uniformly covered with short and long bristles.

Spinnerets (Fig. 3E)*:* PMS digitiform, covered with black hair; PLS covered with black hair, apical segment domed, ventrally with band of spigots (two types), basal segment with short band on distal edge, middle segment with long band for length, apical segment with hairs only on periphery, rest covered with spigots.

Spermathecae (Fig. 3F)*:* paired lobes, each stalk, straight, slightly broader towards base, distally gradually narrowing down with globular apical swollen lobe, at base of lobe, stalk with sclerotized ring twisted band.

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Molecular phylogeny, biogeographic history, and evolution of cave-dwelling taxa in the European harvestman genus *Ischyropsalis* (Opiliones: Dyspnoi)

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Abstract. We estimated a multigenic molecular phylogeny and reconstructed biogeographic history for the European harvestman genus *Ischyropsalis* C.L. Koch 1839 (Dyspnoi). To reconstruct historical biogeographic patterns we conducted an algorithmic VIP analysis which revealed patterns consistent with a vicariance-dominated history. The existing morphology-based systematic framework for *Ischyropsalis* is mostly inconsistent with molecular phylogenetic results, and a new informal system is established that recognizes three main clades and several sub-clades. Species-level analyses revealed two non-monophyletic species (*I. pyrenaea* Simon 1872 and *I. luteipes* Simon 1872); subspecies of *I. pyrenaea* are distant relatives, and are formally elevated to species (*I. pyrenaea pyrenaea* to *I. pyrenaea* and *I. pyrenaea alpinula* to *I. alpinula*). A preference for cryophilic microhabitats has favored the diversification of high-altitude and cave-dwelling *Ischyropsalis* species; molecular phylogenetic data suggest that cave-dwelling species have evolved multiple times independently.

Keywords: Species tree, multilocus phylogeny, biogeographic modelling, convergence, ecological specialization, cave evolution

Modern biogeographic studies of harvestmen have revealed an interesting mixture of evidence for extremely limited dispersal (e.g., Thomas & Hedin 2008; Derkarabetian et al. 2011; Boyer & Reuter 2012), combined with evidence for occasional long-distance dispersal (e.g., Shultz & Regier 2009; Sharma & Giribet 2012; Schönhofer et al. 2013). As such, harvestmen are compelling systems for biogeographic research, but as expected many harvestmen taxa remain to be investigated using modern phylogenetic and biogeographic methods. Within the European fauna, members of the genus *Ischyropsalis* C.L. Koch 1839 are among the most charismatic harvestmen, with relatively large body sizes and massively enlarged chelicerae used to catch prey (Fig. 1). Ecologically, members of this genus are often rare and found in difficult-to-access habitats, including several obligate cave-dwelling species.

Ischyropsalis species limits have been investigated in the context of the biological species concept (Mayr 1942), rarely explicitly applied within arachnids. Martens (1969a) observed *Ischyropsalis* males offering females a secretion from the basal cheliceral segment during courtship and copulation, and demonstrated an association between the secretion offering and subsequent contact between this secretion area and the female mouthparts (Martens 1969b). The male cheliceral secretion area includes bristle fields (Fig. 1) that are connected by minute pores to massive glands (Martens & Schawaller 1977). Martens (1969b) made this cheliceral secretion area and the resulting associated male/female interaction a fundamental character to delineate *Ischyropsalis* species, and also found corresponding male genital characteristics. The presence of such character combinations has also been adopted for species delimitation and higher-level classification in other Opiliones, for example in the Nemastomatidae (Schönhofer & Martens 2012), Dicranolasmatidae (Gruber 1998) and Sabaconidae (Martens 1972; Suzuki 1974).

Studies of *Ischryopsalis* classification began with Hadži (1931), who delineated a single *Ischyropsalis* subgenus, *Odontopalpa,* based on apophysis characters on the male palpal patella (Fig. 1). Originally containing three species, two species were synonymised (Martens 1969b) with *I. kollari* C.L. Koch 1839 (*I. bosnica* Roewer 1914, *I. triglavenis* Hadži 1931), the type species of *Ischyropsalis*. As it was not considered in *Odontopalpa*, the subgenus then became invalid. Hadži originally also placed *I. dentipalpis* Canestrini 1872 and later *I. ravasinii* Hadži 1942 within *Odontopalpa* but had no males to confirm the male-specific characters of this subgenus for the latter. Roewer (1950) described numerous new *Ischyropsalis* species, but was suspected to have separated specimens of common species to describe new species. Martens (1969b) detected these manipulations, later substantiated by v. Helversen and Martens (1972), and synonymized 33 of 34 of Roewer's species.

Morphology-based phylogenetic hypotheses for *Ischyropsalis* were published nearly simultaneously by Martens (1969b) and Dresco (1970). Dresco drew his conclusions from the Paris Museum collection, containing mostly Iberian *Ischyropsalis*, while he had limited access to and knowledge of the remaining European fauna. Martens (1969b) considered most currently-accepted species, and provided an extensive revision based on characters newly developed for *Ischyropsalis* systematics. The systems of Martens and Dresco only agree in the isolated position of *I. hellwigii* (Panzer 1794) while other hypothesised groupings show little congruence (Fig. 2). Authors after 1970 did not comment on the phylogenetic structure of the genus, but added new species or further clarified species synonymies. Martens (1978) refined his system for some Alpine species. Luque (1991, 1992) and Prieto (1990a, b) described the Spanish fauna in more detail. Most recently, Schönhofer and Martens (2010a) and Luque and Labrada (2012) added two new species and discussed regional species diversity in the

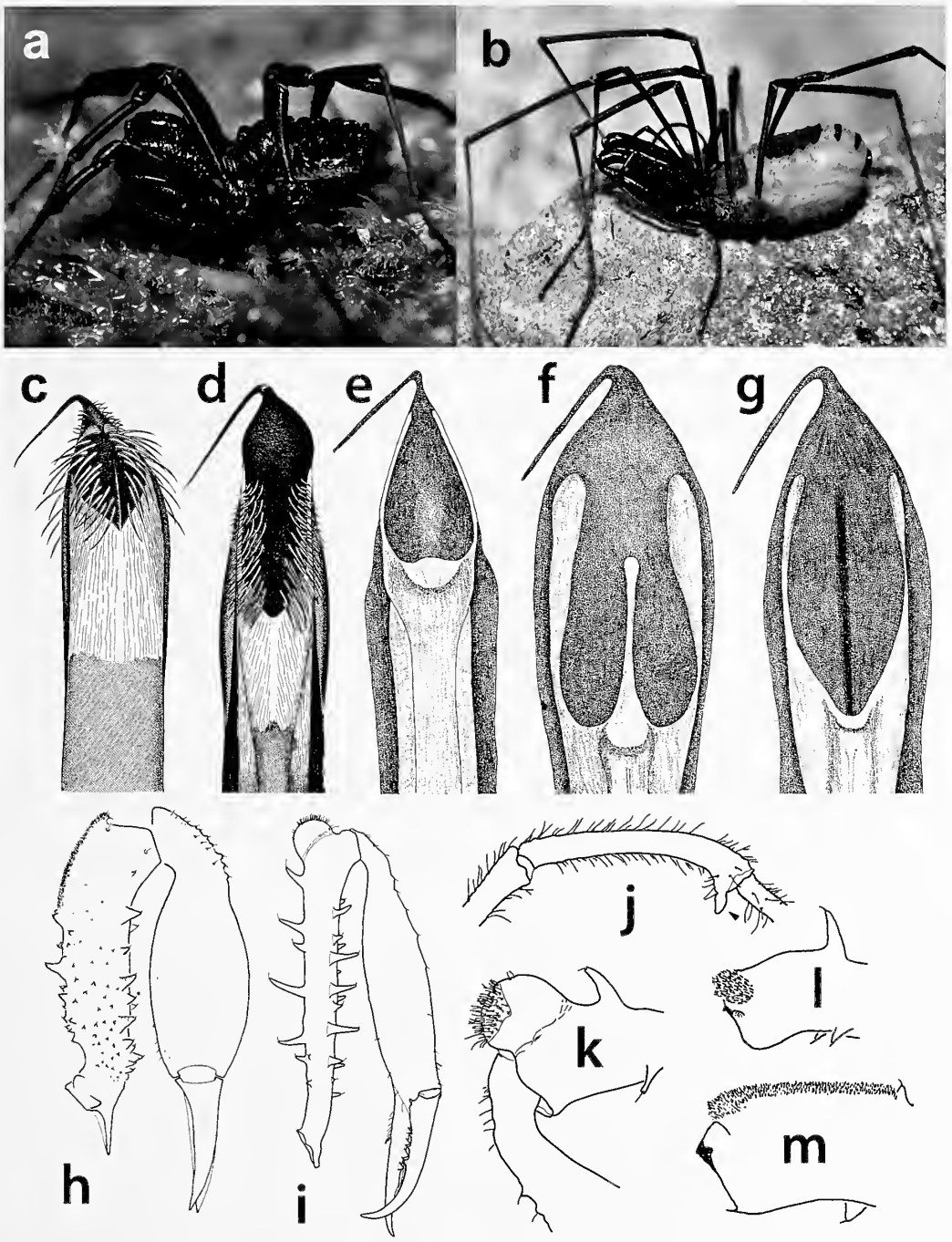

Figure 1.—*Ischyropsalis* habitus and morphological features. a) *I. hellwigii hellwigii* male habitus, Austria, Soboth; b) *I. pyrenaea pyrenaea* female habitus, France, Grotte de l'Estelas; c) distal part of penis, *I. pyrenaea alpinula* (Martens 1978; Fig. 381); d) distal part of penis, *I. dentipalpis* (as *I. helvetica*; from Martens 1978: Fig. 382); e–g) different types of glans sclerites (groups *sensu* Martens, 1969b; Fig. 32): e) *I. hellwigii* group, f) *I. kollari* group, g) *I. dentipalpis* group; h, i) male cheliceral armament, h) *I. hellwigii hellwigii* (Martens 1978: Fig. 289), i) *I. dentipalpis* (Schönhofer & Martens 2010a); j) apophysis on palpal patella of male *I. dentipalpis* (Schönhofer & Martens 2010a; Fig. 6); k–m) distal part of male chelicerae (medial view), apophyses and cheliceral secretion-extruding-field indicated by bristlefield: k) *I. lithoclasica* (Schönhofer & Martens 2010a; Fig. 13), l) *I. adamii*, m) *I. hellwigii* (both Martens 1969b; Fig. 22).

Western Alps and the Cantabrian Mountains, respectively. Twenty-two species-level taxa are currently recognized in the genus (Schönhofer 2013a).

Based on the revision of Martens (1969b), distributional patterns in *Ischyropsalis* suggest a complex biogeographic history. The three species groups of Martens (1969b) are widespread and overlap in distribution, while species within groups are strictly allopatric. This broad-scale phylogenetic structure and the proposed species inter-relationships imply repeated dispersal and isolation between the major mountain systems in Western and Central Europe. On the other hand, individual *Ischyropsalis* species have generally narrow distributions, and at least nine species are obligate cave endemics. Cave-dwelling species are unlikely to disperse over large

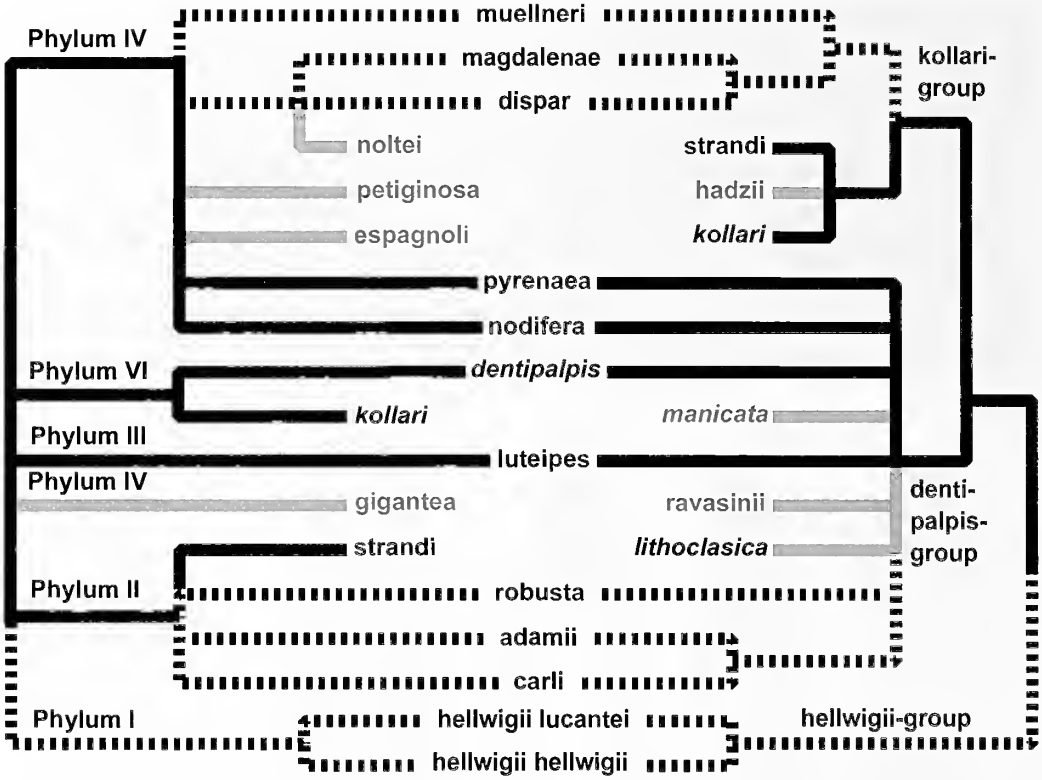

Figure 2.—Morphology-based phylogenetic hypotheses for *Ischyropsalis* according to Dresco (1970, left) and Martens (1969b, right). Some synonymies / misinterpretations used by Dresco were replaced for ease of comparison (we use *I. muellneri* for *I. hellwigii*, *I. hellwigii hellwigii* for *I. taunica*, *I. adamii* for *I. apuanus*, *I. kollari* for *I. trigalvensis* and *I. hellwigii lucantei* for *I. superba*). Phylogenetic lineages are compared and matched if their taxa composition shows at least two matches agreed upon by both authors; matching taxa are indicated on dashed branches; taxa corresponding to alternative lineages are indicated on black branches; taxa considered by only one author are designated in grey. Non-matching species are separated by space between the species names. Species of subgenus *Odontopalpa* sensu Hadži (1931) shown in italics.

distances, and may have originated from widespread epigean species (Porter 2007).

We present here the first molecular phylogenetic analysis of *Ischyropsalis* to investigate the validity of alternative higher-level classification schemes as well as multiple species and subspecies hypotheses (Martens 1969b; Dresco 1970). This molecular phylogenetic framework also allows us to address the phylogenetic value of currently used morphological characters—because of their relatively simple organization and structure, Martens (1969b) suggested the possibility of morphological convergence in male genitalia of *Ischyropsalis*. The distribution of all *Ischyropsalis* taxa is reconstructed based upon literature, museum collections and personal collection data, and these data are used in algorithmic analyses to reconstruct *Ischyropsalis* biogeographic history. Finally, molecular phylogenetic results provide insight into whether cave-dwelling taxa originate from a single ancestor, or have evolved independently multiple times.

METHODS

Sampling.—*Ischyropsalis* specimens were collected by hand, mainly during our own field excursions. Some specimens were also generously donated by colleagues (see Acknowledgments). Specimens were preserved in 100% EtOH, with the majority being collected in 80% and transferred soon thereafter. Morphological identifications were based on Martens (1969b, 1978), Luque (1991, 1992) and Schönhofer and Martens (2010a), or provided by C. Prieto for Spanish samples. A few specimens were raised to adulthood in captivity to allow morphological species identification. We included as many described species as possible, and conducted intraspecific sampling to include geographically and morphologically distinct lineages. Voucher specimen details and repository information are provided in the Appendix. Voucher specimens (with associated collection numbers) are deposited in the collections of J. Martens (CJM, Mainz, Germany), C. Prieto (CCP, Bilbao, Spain), A. Schönhofer (AXLS, Mainz, Germany), C. Vernesi (FEM: Centre for Research and Innovation—Fondazione Edmund Mach, San Michele all'Adige, Italy) and the San Diego State University Terrestrial Arthropod collection (OP).

Molecular methods and analyses.—Genomic DNA was extracted from tissue using the Qiagen DNeasy kit (Valencia, CA, USA). The polymerase chain reaction (PCR) was used to amplify the following gene fragments: 28S rRNA (28S), using the primers ZX1elong (Schönhofer et al. 2013), ZX1, and ZR2 (Mallatt & Sullivan 1998); mitochondrial Cytochrome Oxidase 1 (CO1), using C1-J-1718SPIDERA and C1-N-2776SPIDER (Vink et al. 2005); and nuclear Elongation Factor 1-alpha (EF1α), using OP2BSAB and OPRC4 (Hedin et al. 2010). PCR protocols followed Hedin et al. (2010) for EF1α, Thomas and Hedin (2008) for CO1, and Hedin and Thomas (2010) for 28S, the latter using an annealing temperature of 56°C.

Amplicons were purified on Millipore plates and directly Sanger sequenced at Macrogen USA in both directions. SEQUENCHER v4.5 was used to assemble and edit sequence contigs, with ambiguous sites scored using standard ambiguity codes.

CO1 and EF1α exon sequences were aligned manually in MEGA 4.0 (Tamura et al. 2007) using amino acid translation, while 28S and EF1α intron sequences were aligned with MAFFT vers. 6 (http://mafft.cbrc.jp/alignment/software/), using the Q-INS-i strategy as recommended by Katoh and Toh (2008). To account for alignment uncertainty in the 28S and the EF1α intron data, outgroup genera were removed and the data were realigned using the same strategies as above. Models of DNA sequence evolution were evaluated using jModelTest 0.1.1 (Posada 2008) under three substitution schemes (JC, HKY, GTR) on a fixed BIONJ tree, allowing for unequal base frequencies and among-site rate variation. Final model selection was based on the Akaike Information Criterion (AIC) and individual models were applied to respective partitions in all downstream phylogenetic analyses.

Bayesian inference using MrBayes v3.2.0 (Huelsenbeck & Ronquist 2001; Ronquist et al. 2011) was applied to single genes as well as concatenated datasets. DNA sequences were partitioned by gene, EF1α by exon and intron, and CO1 and EF1α exons by codon position. Bayesian analyses were run for 5 million generations, where in all cases the standard deviation of split frequencies had dropped below 0.01 and standard convergence diagnostics were satisfied for all sampled parameters (ESS >200, PSRF = 1.00; Ronquist et al. 2011). Analyses were repeated to further check for convergence. The first 40% of trees were discarded as burn-in, with remaining trees used to reconstruct a maximum clade credibility tree. Split frequencies were interpreted as posterior probabilities (pp) of clades. A maximum likelihood (ML) concatenated tree using the partitioning scheme outlined above was reconstructed with the raxmlGUI (Stamatakis 2006; Silvestro & Michalak 2012), with support assessed using 1000 rapid bootstrap replications.

As an alternative to concatenation, a species tree was reconstructed using the multispecies coalescent model implemented in *BEAST (Heled & Drummond 2010; Drummond et al. 2012). Models of molecular evolution suggested by jModelTest were implemented, with codon partitioning applied to CO1 and EF1α exons ((1+2), 3). Priors were set to gamma if modified, otherwise default priors were used. Analyses were run until ESS values exceeded 200 for most priors, which was after 200,000,000 generations. Analyses were replicated twice and checked for convergence using Tracer v1.5 (Rambaut & Drummond 2007).

Species trees were estimated using two different sets of operational taxonomic units (OTUs). The first set included only described and accepted species and subspecies (= conservative species set), while the second set was extended to include additional genetic lineages within the species *I. adamii* Canestrini 1873, *I. dentipalpis* and *I. luteipes* Simon 1872 recovered in concatenated analyses (= diversified species set). Preference for one of these OTU settings was evaluated by comparing marginal likelihoods using Bayes Factor analysis (Grummer et al. 2014), as implemented in Tracer v1.5 (Rambaut & Drummond 2007).

Biogeographic analysis.—We compiled geographic distribution data for all known *Ischyropsalis* taxa. A total of 1,360 distribution records were extracted from literature, museum and personal collections, of which 1,220 could be georeferenced at a minimum accuracy of two digital degrees. Over 1,100 coordinates matched species and geographic lineages in our dataset and were used for biogeographic reconstruction. An interactive map with all referenced localities is available online (Schönhofer 2013b).

Applying the extant distribution of species and the species tree derived from *BEAST analyses, the Vicariance Inference Program (VIP, Arias 2010; Arias et al. 2011) was used to infer the distribution of clades and distributional barriers. This program reconstructs the probability of distributional barriers using parsimony. For area reconstruction in VIP the grid was set to 0.3 without fill. To account for misidentifications, a maximum area overlap of 5% was allowed between species. A heuristic search was conducted using 1000 iterations, setting full sector search and otherwise using default settings.

RESULTS

Molecular phylogeny.—A total of 39 *Ischyropsalis* specimens were included in molecular phylogenetic analyses, with samples available for all described subspecies, and 18 of 22 currently described species. Previously published sequences were included from GenBank for *I. luteipes*, *I. robusta* Simon 1872 and *I. nodifera* Simon 1879 (Shultz & Regier 2001; Schönhofer & Martens 2010b; Giribet et al. 2010; see Appendix). Outgroup sequences for the sister genera *Ceratolasma* Goodnight & Goodnight 1942 and *Acuclavella* Shear 1986 were obtained from Richart & Hedin (2013).

The protein alignments of CO1 and the EFIα exon contained no gaps and no stop codons. We note that although EFIα paralogs have been detected in mite harvestmen (Clouse et al. 2013), we found no evidence for paralogs in *Ischyropsalis*. PCR products directly Sanger sequenced showed no evidence for multiple overlapping sequences as would be expected if paralogs were common. However, this result is potentially biased by the PCR primers that we utilized. As such, we also searched for EFIα paralogs in the transcriptomes of other ischryopsalidoid taxa (Hedin et al. 2012; unpublished data). Specifically, we blasted *Ischyropsalis* EFIα sequences (*I. carli* Lessert 1905, AXLS 145) against the transcriptomes of *Sabacon* Simon 1879, *Acuclavella* and *Hesperonemastoma* Gruber 1970 (tblastx, e value 1e-20) in Geneious 7.0.6. These searches recovered a single EFIα sequence for each of these ischryopsalidoid taxa, suggesting a lack of paralogy in this clade. We emphasize that Opiliones is an old taxon with ancient internal divergences (Hedin et al. 2012), and that gene duplication in one lineage does not imply similar molecular evolutionary patterns in other, distantly related taxa. Similar dynamics of EFIα paralogy gain and loss are observed, for example, in insects (Djernaes & Damgaard 2006).

Models of sequence evolution were selected as follows: 28SrRNA: GTR+I+G; COI codon position 1: SYM+G; COI codon position 2: F81+G; COI codon position 3: GTR+G; EFIα codon position 1: HKY+I+G; EFIα codon position 2: HKY; EFIα codon position 3: GTR+G; EFIα intron: HKY+G. In *BEAST settings were modified for EFIα-exon to SYM+I+G (in *BEAST set to TN93+I+G, base frequencies

equal) and for COI to GTR+I+G (same in *BEAST), both separated by codon.

Ischyropsalis is clearly monophyletic with respect to the North American *Acuclavella* and *Ceratolasma*, allowing removal of these outgroups for further reconstructions. Results of the concatenated Bayesian and ML analyses, both with and without outgroups, were generally congruent (Fig. 3), with the few topological differences observed at weakly supported nodes. Alternative topologies recovered different arrangements between *Ischyropsalis lithoclasica* Schönhofer & Martens 2010a, *I. kollari* and *I. ravasinii*, and grouped *I. navarrensis* Roewer 1950 with *I. robusta* in the ML analyses. The same was true for the position of terminals within the two *I. luteipes* clades and *I. carli* (Fig. 3).

Bayesian and ML molecular phylogenetic results indicate that *Ischyropsalis* is divided into three primary clades, defined here for further discussion: 1) the *I. hellwigii* group (comprising the two described subspecies), 2) the *I. manicata* group (including *I. adamii*, *I. carli*, *I. manicata* L. Koch 1869 and *I. pyrenaea alpinula* Martens 1978), and 3) the Iberian-Alpine group, including all remaining sampled species. The composition of these groups is further detailed in the Discussion. Molecular phylogenetic support for these clades is strong (e.g., Bayesian posterior probability values > 0.95), although slightly lower for the *I. manicata* group, whieh includes several species that are missing data for multiple genes (Appendix).

Bayesian and ML concatenated analyses (Fig. 3) recovered most of the currently accepted species as well-supported lineages, with some exceptions. The subspecies of *Ischyropsalis pyrenaea* are not recovered as monophyletic, but instead fall into different higher-level groups. *Ischyropsalis luteipes* was found to be paraphyletic with *I. pyrenaea pyrenaea* nested within *I. luteipes*. Intraspecific genetic divergences were generally low, except for *I. adamii* and *I. dentipalpis*, both including divergent genetic lineages. Distinct lineages within the non-monophyletic or internally-divergent taxa were defined as distinct OTUs in the *BEAST analysis of the diversified species set, and all received strong support (Fig. 4). However, the Bayes Factor analysis did not conclusively favor one species-set hypothesis over the alternative hypothesis (ln Bayes Factor: 3.635).

Biogeographic analysis.—VIP results are visualised in Figs. 5 and 6, including distributions of all higher clades (Fig. 5) and the Iberian-Alpine-group (Fig. 6). VIP recognized thirteen barriers corresponding to phylogenetic splits. Barriers were found in consistent positions over all reconstructions, except for a few cases when individual VIP reconstructions dropped taxa, which shifted the barrier in one direction towards one of the included groups but never changed its directionality or general position.

Biogeographic analyses indicate few putative long-range dispersal events between different European mountain systems within the *I. hellwigii* and *I. manicata* groups. The Iberian-Alpine group showed a general pattern of diversification coinciding with the division of ancestral areas, resulting in decreasing distribution areas with decreasing phylogenetic age of the clades, with evidence for long-range dispersal generally absent. Thus geographic proximity generally coincides with closer phylogenetic relationship within the Iberian-Alpine group (Fig. 6). Species distributions within the Iberian-Alpine group are not strictly allopatric but overlap in many cases, and in the Iberian distributional area at least two sympatric sister species are present.

DISCUSSION

Higher-level phylogeny.—Comparing phylogenetic hypotheses (Fig. 2) of Martens (1969b) and Dresco (1970) with molecular-based concatenated (Fig. 3) or *BEAST trees (Fig. 4) reveals little congruence. These alternative hypotheses agree only in the separate (Martens 1969b; Dresco 1970) and basal placement (Martens 1969b) of the *I. hellwigii* group. Further similarities only partly coincide with para- and polyphyletic groups, except for Hadži's *Odontopalpa*, that receives weak support in our analyses. Hadži (1931) included the species *I. kollari* (sub *I. triglavensis*) and *I. dentipalpis* (presently split into *I. lithoclasica* and *I. dentipalpis* sensu stricto; Schönhofer & Martens 2010a) in this subgenus and assumed *I. ravasinii* to be included, although he had no males to confirm this placement. Martens (1969b) considered the genus *Ischyropsalis* too small to justify establishment of subgenera. Accepting *Odontopalpa* would require the definition of several additional subgenera, which he felt carried little taxonomic value—we generally agree with this recommendation.

While the detailed studies of previous authors refined the characters for species-level taxonomy, higher-level groups (as implied by molecular phylogeny) now lack morphologically diagnostic characters. This situation is similar to other harvestmen groups, for example *Trogulus* Latreille 1802, where characters delineating species appear clearer than those characterizing higher-level groups (Schönhofer & Martens 2010b). The molecular results for *Ischyropsalis* suggest that most of the presently used morphological systematic characters are randomly distributed throughout the phylogeny. Even male-specific characters of the genitalia and chelieeral apophyses seem problematic for use as phylogenetic characters. This is not completely unexpected as Martens (1969b) mentioned the relatively simple organization of the penis in *Ischyropsalis*, perhaps prone to homoplasy or morphological stasis.

Character conflict is well-illustrated by the "subspecies" of *I. pyrenaea*, previously believed to be closely related based on morphology, but apparently belonging to different groups within *Ischyropsalis* (Fig. 3). The proposed shared characters of the *I. pyrenaea* subspecies (Martens 1969b, 1978), including cheliceral spination and apophyses, the male cheliceral bristle field and the overall similarity of the male genital morphology (e.g., un-lobed and keeled sclerites of the penial glans), are likely convergent. Indeed, the structure of these sclerites differs considerably in detail, particularly in the connection towards the sclerotised part of the penial stylus (Fig. 1). In *I. pyrenaea pyrenaea* this sclerite is rather broad, while in *I. pyrenaea alpinula* it is extremely narrow, appearing almost petiolate.

An overview of characters for diagnosing recovered molecular clades is given below. *Ischyropsalis hadzii* Roewer 1950, *I. gigantea* Dresco 1968, *I. petiginosa* Simon 1913 and *I. cantabrica* Luque & Labrada 2012, not included in our data set, are also assigned tentatively to these larger groups.

I) *I. hellwigii* group (two described subspecies *I. h. hellwigii*, *I. h. lucantei* Simon 1879): Chelicerae massive, males with

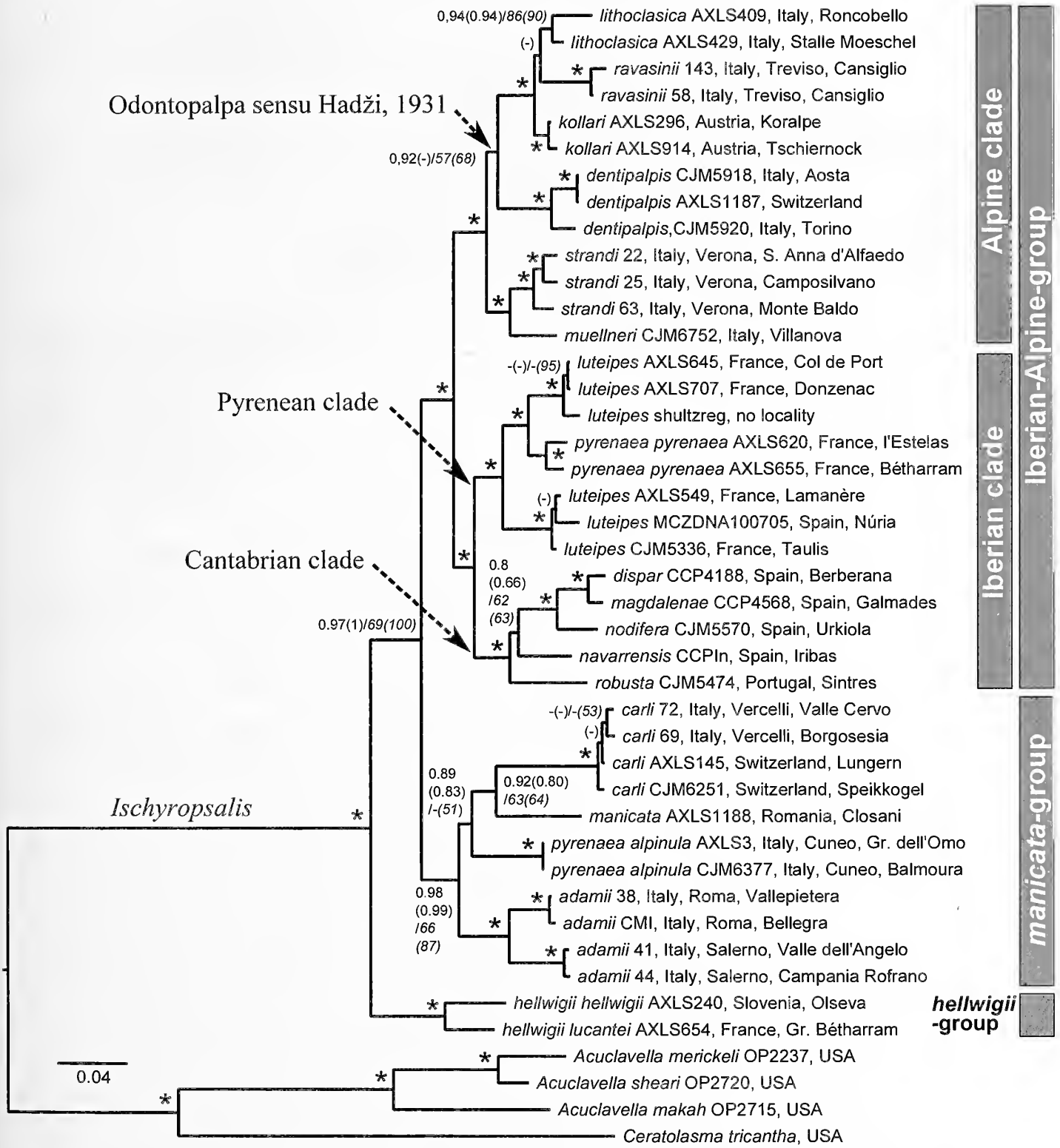

Figure 3.—Bayesian consensus phylogram based on concatenated molecular data. Numbers at nodes show support values resulting from different analyses: 1) Bayesian posterior probabilities; numbers in parentheses are Bayesian posterior probabilities after removal of outgroup genera and realignment of 28S and EF1α intron, 2) maximum likelihood bootstrap values (in italics); in parentheses after removal of outgroup and realignment of 28S and EF1α intron. Nodes with pp = 1.00 and bootstrap values >94 indicated by asterisks. Support values below 0.94 pp and 70 bootstrap not shown.

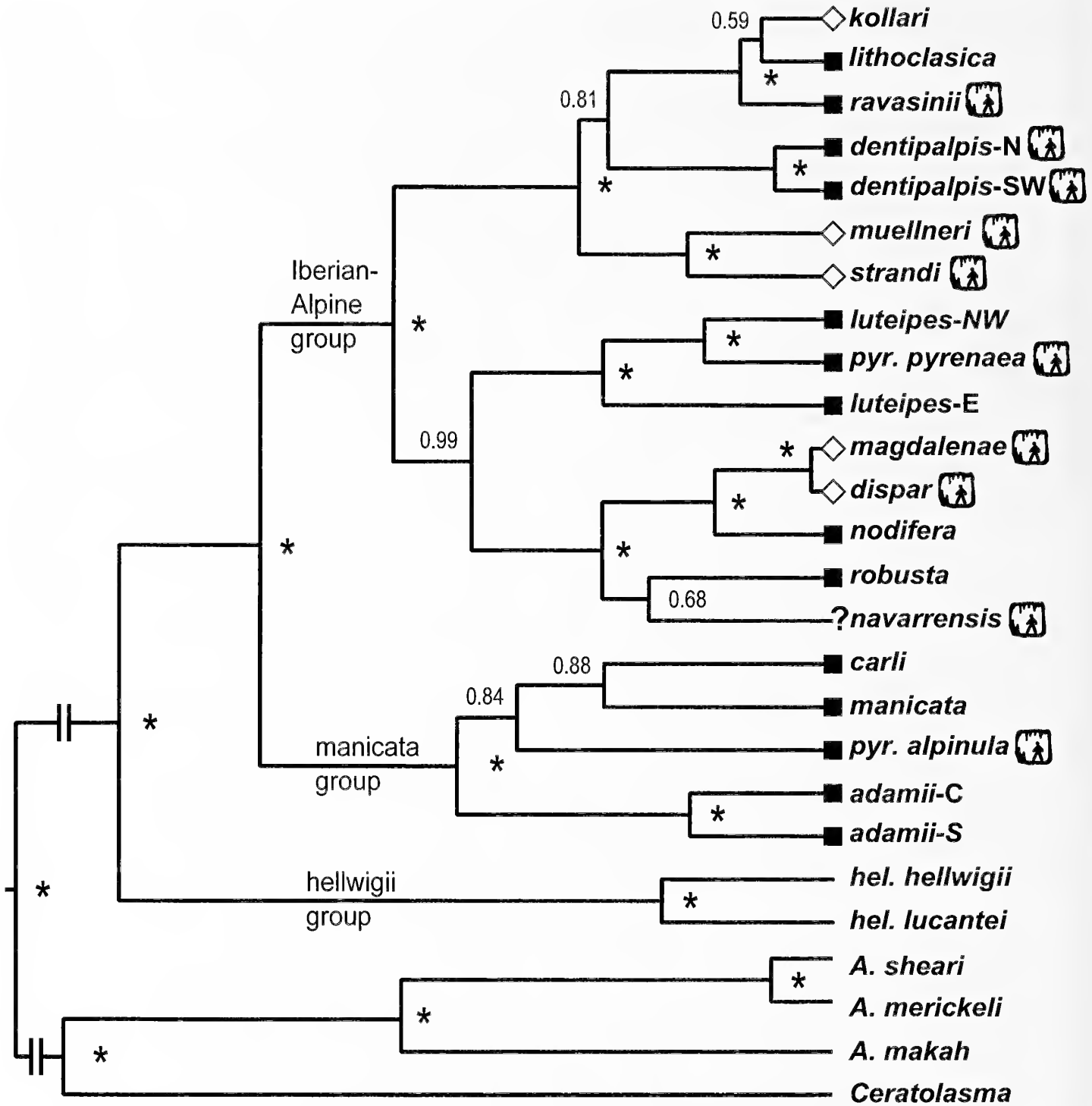

Figure 4.—*BEAST species tree. Basal nodes graphically shortened to allow expanded view of ingroup. Posterior probabilities given at nodes; pp of 1.00 indicated by an asterisk. Distribution of species group taxa according to Martens (1969b) is symbolized at tips: *dentipalpis* group indicated by black squares, *kollari* group indicated by open diamonds. *I. lithoclasica* was formerly considered *I. dentipalpis*, *I. dentipalpis* was considered *I. helvetica* (Martens 1969b; 1978), and *I. pyrenaea alpinula* was described as a subspecies by Martens (1978). Predominantly cave-dwelling species indicated by a cave icon.

a large dorsal cheliceral glandular bristle field. Sclerite of penial gland small, with irregularly spaced small spines. *Ischyropsalis h. lucantei* is restricted to the central Pyrenees, while *I. h. hellwigii* occupies a large area from Germany to Southern Poland.

The following groups share long bristles on the penial glandes:

II) *I. manicata* group (*I. adamii*, *I. carli*, *I. manicata*, *I. pyrenaea alpinula*): Penial sclerites narrow-stalked, appearing almost narrow leaf- to broad heart-shaped.

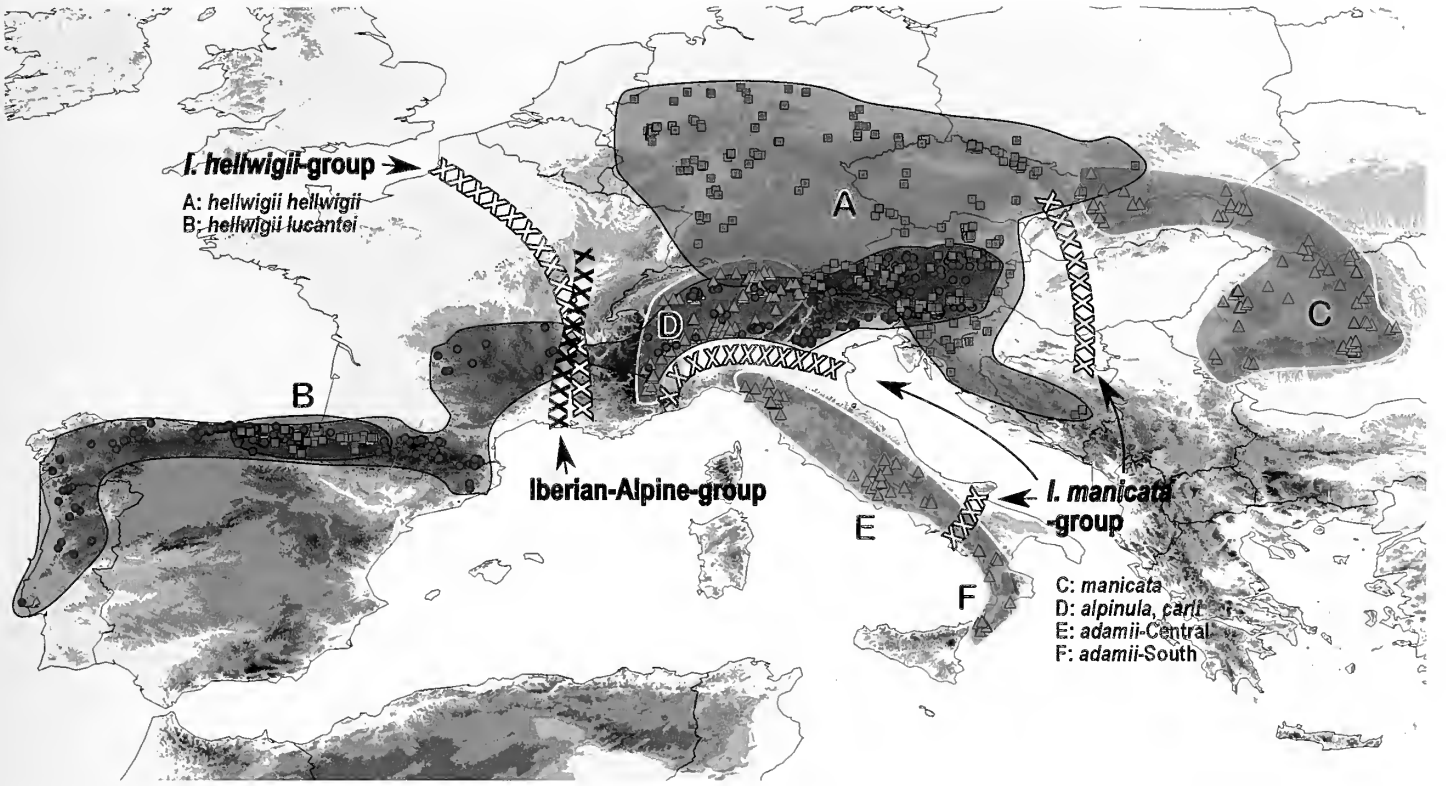

Figure 5.—Distribution of primary *Ischyropsalis* groups in Europe, with VIP barriers shown as lines of crosses. Major groups include the *I. hellwigii* (green squares), *I. manicata* (yellow triangles), and Iberian-Alpine (red circles) groups.

Sclerites always keeled and never bilobed, males with small distinctly circumscribed distolateral cheliceral glandular bristle field. Individual species have a rather widespread distribution. Two species in the western Alps (*I. carli*, *I. pyrenaea alpinula*), and one species in the Carpathians (*I. manicata*) and Apennines (*I. adamii*), respectively; in the latter two areas these represent the only *Ischyropsalis* taxa known.

III) Iberian-Alpine group: Including the remaining species. Penial sclerites broad-stalked or not narrowed towards intersection with stylus. Otherwise morphologically heterogeneous and best separated by geographic distribution.

IIIa) Alpine-clade: the group is separated into two morphologically separable elades that share few characteristics, maybe the bilobed penial sclerite, which is not split in *I. dentipalpis*. Most species of the first group (*I. dentipalpis*, *I. kollari*, *I. lithoclasica*, *I. muelhneri* Hamann 1898) exhibit a unique patellar apophysis on the male palp (missing in *I. hadzii* that likely belongs to this group as well) and have a large plateau-like bristle-field with sparse and scattered setation on the basal cheliceral segment (also in *I. hadzii*). Members of the second group within this clade (*I. ravasinii* and *I. strandi* Kratochvíl 1936) lack the palpal apophyses and bristle field and apophyses are reduced or missing. Both groups are distributed mainly in the Central to Eastern Alps, with only *I. dentipalpis* in the Western Alps.

IIIb) Iberian-clade (including two subclades): many species have keeled penial sclerites, otherwise sclerites are always deeply bi-lobed. Males have large triangular dorsodistal cheliceral apophyses. Members of the Pyrenean clade (*I. luteipes* [two lineages], *I. pyrenaea pyrenaea*) share no perceivable characters, which highlights that most observed character states can evolve rapidly. Uniting features include distribution in the Pyrenees and molecular synapomorphies. The Cantabrian clade (*I. dispar* Simon 1872, *I. magdalenae* Simon 1881, *I. navarrensis*, *I. nodifera*, *I. robusta*, *I. petiginosa*, likely *I. cantabrica* and *I. gigantea*) has a primary uniting character, the bristle field on the basal cheliceral segment, situated on the distomedial side. The cheliceral apophysis is generally very large, often triangular and pointed, viewed laterally. This group is in need of further evaluation, including study of more populations, missing species and more refined geographic analyses.

Species-level systematics.—Our molecular phylogenetic results fail to recover two described species as monophyletic, or recover species as monophyletic but with high internal genetic divergence, perhaps consistent with cryptic or incipient speciation. As previously discussed the two subspecies of *Ischyropsalis pyrenaea* are distantly related (Figs. 3 & 4). Considering their phylogenetic distance, they are hereby formally elevated to species (*I. pyrenaea pyrenaea* to *I. pyrenaea* and *I. pyrenaea alpinula* to *I. alpinula*). A careful redescription is warranted and may reveal further phyloge-

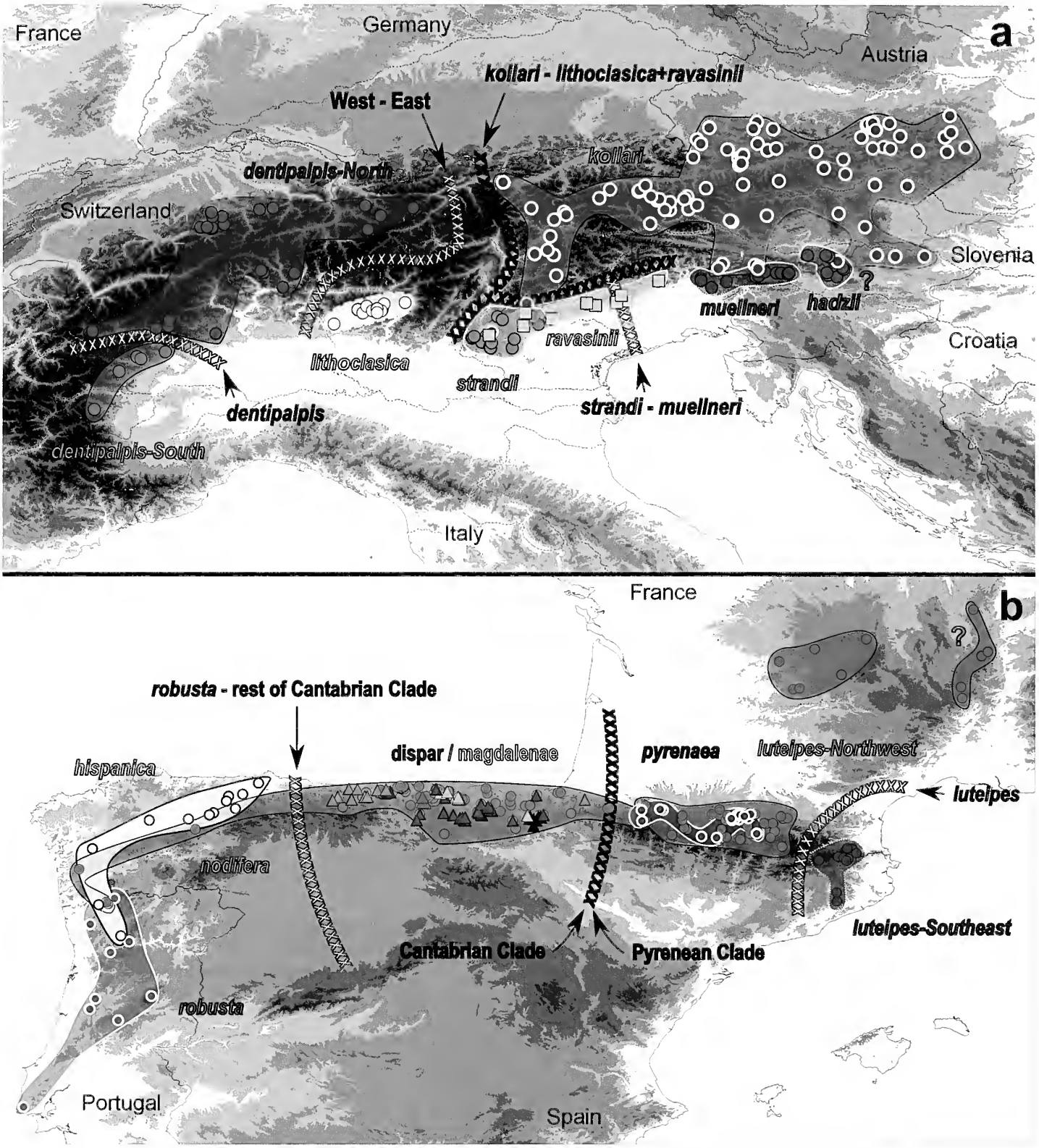

Figure 6.—a. Distribution of the *Ischyropsalis* Alpine clade in the Alps showing biogeographic reconstructions within major clades; b. Distribution map of the *Ischyropsalis* Iberian clade on the northern Iberian Peninsula and southern France. The distribution of microendemic species in the Cantabrian Mountains of northern Spain was not further resolved. For both a & b, colors correspond to individual species and lineages as supported by *BEAST and other analyses, and VIP barriers are shown as lines of crosses. Question marks indicate populations or species where phylogenetic information is lacking.

netically useful characters. Also in need of further study is the paraphyletic *I. luteipes*, with the morphologically different *I. pyrenaea* nested within this lineage (Fig. 3). Dresco (1970) discussed three "ecoforms" of *I. luteipes* but did not formally describe these or provide characters for delineation. Character reinvestigation and additional comprehensive geographic sampling is required.

The central and southern lineages of the Italian *Ischyropsalis adamii* are genetically clearly separable (Fig. 3), while material from the isolated northern population was not available for this study. Individual names for all three populations have been proposed by earlier authors and require reassessment (southern Italy: *I. adamii*; northern Italy: *I. apuanus* Caporiacco 1930; central Italy: *I. apuanus nanus* Dresco 1968). Similarly, our data indicate that the southwestern-most population of *I. dentipalpis* is genetically distinct. This species has been recently re-evaluated based on morphology (Schönhofer & Martens 2010a), and further studies of its fragmented distribution were suggested. Our results indicate differentiation within *I. dentipalpis* but refute a close relationship to *I. lithoclasica* as hypothesized by Schönhofer and Martens (2010a).

Historical biogeography.—While Martens (1969b) assumed widespread species groups with strictly allopatric species, our phylogenetic results indicate a clearly different pattern. Herein, diversification and speciation is generally restricted to the same mountain range, specifically the Alps, the Cantabrian Mountains, the Pyrenees, and, to a lesser extent, the Apennines. Only in the monotypic *Ischyropsalis hellwigii* and *I. manicata* groups are species and lineages distantly allopatric, consistent with old vicariance or long-range dispersal (Fig. 5). The remaining *Ischyropsalis* species belonging to the Iberian-Alpine group are frequently sympatric, sometimes with completely overlapping distributions of closely related taxa, e.g., *I. luteipes* and *I. pyrenaea*. The biogeographic pattern within the Iberian-Alpine group shows a general trend of decreasing distribution area with decreasing taxonomic level, with the distribution of descendants contained within the distributional area of reconstructed ancestors. Therefore, dispersal seems not to be the main cause of diversification within *Ischyropsalis*, which remains difficult to test without reliably assignable fossils. The lack of *Ischyropsalis* fossils also means that we cannot rule out the presence of now extinct representatives in areas outside the known distribution of the genus.

Evolution of habitat preferences.—Most extant *Ischyropsalis* species are confined to montane habitats, preferring microhabitats with moderate to cold temperatures and high and constant humidity. On the Iberian Peninsula *Ischyropsalis* species are confined to mountain regions situated along the northern and western coast, characterised by high and consistent rainfall and moderate variation in annual temperatures (Immerzeel et al. 2009). Precipitation in the Alps is generally high, and low temperature microhabitats are abundant at high altitudes in this mountain chain.

It is noticeable that *Ischyropsalis* shows a general trend from more generalist and widespread species (*I. hellwigii* and *I. manicata* groups) towards specialized short-range endemics (most members of the Iberian-Alpine group). This trend manifests in the high number of cave endemics in *Ischyropsalis*, which may be considered extreme ecological specialists. Molecular phylogenetic trees include no large clades of exclusively cave-dwelling taxa (Fig. 4). Rather, it appears that cave species have evolved multiple times in clades of mainly epigean species, a pattern reported from many other cave radiations (Porter 2007), including harvestmen (Derkarabetian et al. 2010; Hedin & Thomas 2010; Derkarabetian & Hedin 2014). However, the degree of cave specialization in *Ischyropsalis* is variable, with many surface-dwelling species regularly entering caves. Caves are the only available habitats at low altitudes, with surface habitats such as alpine gravel or forests increasing towards high altitudes. Fully troglomorphic taxa are rare, as most species retain eyes. The tendency towards troglomorphism correlates with a preference for cooler and more stable temperatures. For example, temperature preferences for the surface-dwelling *I. luteipes* were recorded at 11.5 °C with a range of 10 °C (Juberthie 1964). In the troglomorphic *I. pyrenaea pyrenaea* the mean temperature is comparable, but the range does not exceed 5 °C. A similar preference range is exhibited by eastern Alpine cave endemics but with mean preferences shifted to even lower temperatures (*I. strandi*: 3.5–7.2 °C; *I. muellneri*: 5.2–7.8 °C; Juberthie 1964). The frequent evolution of cave specialization enables the regional co-occurrence of epigean and predominantly troglomorphic species. The preference for low temperatures might also explain the general absence of *Ischyropsalis* in more southern cave systems as on the Balkan, as these caves generally show higher mean temperatures.

Members of the Iberian-Alpine group and all cave endemic species may be vulnerable to climate change given their geographically restricted ranges and narrow ecological preferences. Considering these factors, it will be important to protect relevant cave habitats as comprehensively as possible.

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Appendix.—Specimens included in phylogenetic analyses.

Organism	Voucher	Locality	Lat (N)	Long (E)	28S acc.-no.	COI acc.-no.	EF1a acc.-no.
Acuclavella makah Richart and Hedin 2013	OP2715	USA: Washington, Clallam Co., Hoko Falls	no data	no data	KF181753[1]	KF181737[1]	KF181770[1]
Acuclavella merickeli Shear 1986	OP2237	USA: Idaho, Idaho Co., FS 443 0.8 mi S of Selway Rv Rd	no data	no data	KF181751[1]	KF181733[1]	KF181767[1]
Acuclavella sheari Richart and Hedin 2013	OP2720	USA: Idaho, Idaho Co, FS 592	no data	no data	KF181756[1]	KF181743[1]	KF181777[1]
Ceratolasma tricantha Goodnight and Goodnight 1942	OP989	USA: OR, Curry Co., E. Gold Beach (COI sequence from different specimen[2])	no data	no data	JX573543[2]	GQ912865[3]	JX573601[2]
Ischyropsalis adamii Canestrini 1873	FEM 038/G/2006	Italy: Lazio, Prov. Roma, Vallepietera, Pozzo Cornetto	41.94	13.23	KP224348	KP224375	KP224405
Ischyropsalis adamii Canestrini 1873	Coll. M. Isaia (CMI) I_adamii	Italy: Lazio, Roma, (GPS for) Bellegra, Grotta dell'Arco 5La	41.88	13.03	-	KP224376	KP224406
Ischyropsalis adamii Canestrini 1873	FEM 041/G/2006	Italy: Campania, Salerno, Valle dell'Angelo, Grotta dei Briganti	40.29	15.39	KP224349	KP224377	KP224407
Ischyropsalis adamii Canestrini 1873	FEM 044/G/2006	Italy: Campania, Salerno, Campania Rofrano, Grava di Carlo (del pian delle lepri)	40.21	15.42	KP224350	KP224378	KP224408
Ischyropsalis carli Lessert 1905	FEM 072/G/2006	Italy: Piemonte, Prov. Vercelli, Cavità artificiale alta valle Cervo ex miniera alpe Machetto	45.61	8.04	KP224351	KP224379	KP224409
Ischyropsalis carli Lessert 1905	FEM 069/G/2006	Italy: Piemonte, Prov. Vercelli, Valduggia, Comune di Borgosesia, Grotta delle Arenarie (2509 Pi/VC)	45.714	8.315	-	KP224380	KP224410
Ischyropsalis carli Lessert 1905	AXLS 145	Switzerland: Kanton Oberwalden, Lungern, Wengenhöhle (661245/186818)	46.83	8.24	JX573544[2]	JX573638[2]	JX573602[2]
Ischyropsalis carli Lessert 1905	CJM 6251	Switzerland: Kanton Bern, stream above Gantrischseeli (lake N mountain Gantrisch)	46.70307	7.45150	KP224352	-	-
Ischyropsalis dentipalpis Canestrini 1872	AXLS 1187	Switzerland: Kanton Obwalden, Sachseln, Sachseler Seefeld, Höhle S1 (Swiss-Coordinates 659710 / 182750)	46.793	8.225	-	KP224381	KP224411
Ischyropsalis dentipalpis Canestrini 1872	CJM 5918	Italy: Prov. Aosta, Gressoney, Pozzo A di Punta Jolanda ((Ao/AO 2075)	45.84	7.84	KP224353	KP224382	-
Ischyropsalis dentipalpis Canestrini 1872	CJM 5920	Italy: Piemonte, Prov. Torino, Brosso, Valchiusella-Valley, Buca del Ghiaccio della Cavallaria (Pi/TO 1609)	45.52	7.80	KP224354	KP224383	-
Ischyropsalis dispar Simon 1872	CCP CCP4188	Spain: Burgos, t.m. Villalba de Losa, (Perilde) Berberana, Sierra Salvada, Cueva Albia (VN9-5-)	42.9706	−3.0728	KP224355	KP224384	KP224412
Ischyropsalis hellwigii hellwigii (Panzer 1794)	AXLS 240	Slovenia: Olševa-Mountains, locality Sadni Travniki	46.45708	14.68605	JX573545[2]	JX573639[2]	JX573603[2]
Ischyropsalis hellwigii lucantei Simon 1879	AXLS 654	France: France: Midi-Pyrénées, Dép. Hautes-Pyrénées, Lourdes, Saint-Pé-de-Bigorre, Grottes de Bétharram	43.10234	−0.18478	KP224356	KP224385	KP224413
Ischyropsalis kollari C.L. Koch 1839	AXLS 296	Austria: Steiermark, Koralpe, Wolfsberg, Großer Speikkogel, locality Erlenloch	46.79874	14.95092	JX573546[2]	-	JX573604[2]
Ischyropsalis kollari C.L. Koch 1839	AXLS 914	Austria: Kärnten, Treffling, Tschiernock Mt.	46.85279	13.58791	KP224357	KP224386	KP224414

Appendix.—Continued.

Organism	Voucher	Locality	Lat (N)	Long (E)	28S acc.-no.	COI acc.-no.	EF1a acc.-no.
Ischyropsalis lithoclasica Schönhofer and Martens 2010	AXLS 429	Italy: Prov. Bergamo, Valle Valzurio, Oltressenda Alta, picknick-area W Stalle Möschel	45.93810	10.00446	KP224358	-	KP224415
Ischyropsalis lithoclasica Schönhofer and Martens 2010	AXLS 409	Italy: Prov. Bergamo, Roncobello, Corno Branchino and Lago Branchino	45.94931	9.80257	KP224359	KP224387	KP224416
Ischyropsalis luteipes Simon 1872	AXLS 645	France: Midi-Pyrénées, Dép. Ariège, Col de Port	42.89675	1.44922	KP224360	KP224388	KP224417
Ischyropsalis luteipes Simon 1872	AXLS 707	France: Limousin, Dép. Corrèze, Brive-la-Gaillarde, N Donzenac	45.23460	1.54536	KP224361	KP224389	-
Ischyropsalis luteipes Simon 1872	no data	no data	no data	no data	-	-	AF240870[4]
Ischyropsalis luteipes Simon 1872	Museum of Comparative Zoology 100705	Spain: Girona, Queralps, Núria, Font de l'Home Mort	42.36	2.12	GQ912767[3]	-	-
Ischyropsalis luteipes Simon 1872	CJM 5336	France: Languedoc-Roussillon, Dép. Pyrénées-Orientales, D618 between St.-Marsal und Taulis	42.52925	2.6112	GQ466293[5]	KP224390	KP224418
Ischyropsalis luteipes Simon 1872	AXLS 549	France: Languedoc-Roussillon, Dép. Pyrénées-Orientales, Lamanère	42.35874	2.51685	KP224362	KP224391	KP224419
Ischyropsalis magdalenae Simon 1881	CCP CCP4586	Spain: País Vasco, Biscay, Galmades, Mina la Fragua			KP224363	KP224392	KP224420
Ischyropsalis manicata C.L. Koch 1865	AXLS 1188	Romania: Closani, Ranca, cave	45.08	22.80	-	KP224393	-
Ischyropsalis muellneri Hamann 1898	CJM 6752	Italy: Friuli, Prov. Udine, Luzevera, Villanova, Fr 548, Abisso Mario Grassi	46.25	13.27	KP224364	KP224394	-
Ischyropsalis navarrensis Roewer 1950	CCP CCP I.n.	Spain: Navarra, Sierra de Aralar, Iribas, Sima de Lezegalde	42.986	-1.904	KP224365	KP224395	KP224421
Ischyropsalis nodifera Simon 1879	CJM 5570	Spain: País Vasco, Prov. Álava, ca. 5km S of pass Urkiola	43.0734	-2.6619	KP224366	KP224396	-
Ischyropsalis pyrenaea alpinula Martens 1978	AXLS 3	Italy: Piemonte, Prov. Cuneo, Grotta O-5 dell'Omo	44.3722	7.1374	KP224367	KP224397	KP224422
Ischyropsalis pyrenaea alpinula Martens 1978	CJM 6377	Italy: Piemonte, Prov. Cuneo, betw. Grange Pieccia and Chiappi, Grotta Balmoura	44.4497	7.1502	KP224368	KP224398	-
Ischyropsalis pyrenaea pyrenaea Simon 1872	AXLS 620	France: Midi-Pyrénées, Dép. Ariège, NW Cazavet, Grotte de l'Estelas	42.99665	1.0101	JX573547[2]	JX573641[2]	JX573605[2]
Ischyropsalis pyrenaea pyrenaea Simon 1872	AXLS 655	France: Midi-Pyrénées, Dép. Hautes-Pyrénées, Lourdes, Saint-Pé-de-Bigorre, Grottes de Bétharram	43.10234	-0.18478	KP224369	KP224399	KP224423
Ischyropsalis ravasinii Hadži 1942	FEM 143/G/2006	Italy: Veneto, Prov. Treviso, Pian del Cansiglio, Bus de la Genziana	46.028	12.407	KP224370	KP224400	KP224424
Ischyropsalis ravasinii Hadži 1942	FEM 058/G/2006	Italy: Veneto, Prov. Treviso, Pian del Cansiglio, Bus de la Genziana	46.028	12.407	KP224371	-	KP224425
Ischyropsalis robusta Simon 1872	CJM 5474	Portugal: 20 km W Lissabon, Sintres	38.79325	−9.3929	GQ466294[5]	KP224401	KP224426
Ischyropsalis strandi Kratochvíl 1936	FEM 022/G/2006	Italy: Veneto, Prov. Verona, S. Anna d'Alfaedo, Spluga della Preta	45.678	10.952	KP224372	KP224402	KP224427
Ischyropsalis straudi Kratochvíl 1936	FEM 025/G/2006	Italy: Veneto, Prov. Verona, Camposilvano, Buso dell'Arena	45.62	11.03	KP224373	KP224403	KP224428
Ischyropsalis strandi Kratochvíl 1936	FEM 063/G/2006	Italy: Veneto, Prov. Verona, Monte Baldo, Novezzina Grotta RH9	45.69	10.85	KP224374	KP224404	KP224429

[1] Richart & Hedin 2013; [2]Schönhofer et al. 2013; [3]Giribet et al. 2010; [4]Shultz & Regier 2001; [5]Schönhofer & Martens 2010b.

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Determination of in-lab site fidelity and movement patterns of *Paruroctonus utahensis* (Scorpiones: Vaejovidae)

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Abstract. Many animals build homes to which they return after excursions. However, the sensory and motor mechanisms that animals use to home are poorly understood. Sand scorpions, including *Paruroctonus utahensis* (Williams 1968), make burrows from which they emerge to hunt at night. These scorpions spend most of their surface time within about a meter of their burrow. Our goal was to create a laboratory environment conducive to scorpion homing behavior. Specifically, our objectives were to verify in-lab burrow use similar to field observations and to characterize scorpion movements in these artificial environments. Tests occurred in circular, sand-filled arenas (65 cm diam); in the center of each was a shelter (a small jar lid with openings for the scorpions to enter). We used IR cameras to film all trials from above in a room with a 14:10 hour light-dark cycle. Animals were tested over a 7-day period for their tendency to establish and return to their shelters on a normal day/night cycle. Time-in-shelter percentages showed significant evidence of shelter use, consistent with their normal burrow use in the field. In the second experiment, we wrote a MATLAB program to automatically track several hours of videotaped scorpion nocturnal movements. Animals spent most of their time along the arena walls but made intermittent forays across the arena center. When they returned to their shelters, their movements appeared to be direct and deliberate. This behavioral set-up will be useful in future attempts to deduce the sensory information these animals use to return to their burrows.

Keywords: Homing, behavior, navigation, sensory, scorpion

Many species of animals build homes to which they repeatedly return, suggesting navigational capabilities. While humans use vision, extensive memory, and other methods to navigate, many animals rely on different means. For example, desert ants appear to retrace familiar visual paths learned during training excursions established initially by path integration (Collett & Collett 2000; Wehner & Srinivasan 2003; Wittlinger et al. 2006; Baddeley et al. 2012), some slugs use odors as clues that help them find their homes (Chelazzi et al. 1988), and fiddler crabs often use "kinesthetic short-range homing," a form of path integration, when near their burrows (Cannicci et al. 1999). Other arachnids, such as spiders, have also been shown to orient and navigate using path integration (Moller & Görner 1994; Ortega-Escobar 2002). One problem is that many of the typically studied homing animals (bees, pigeons, bats) travel great distances, making it difficult to track their exact long-range paths in the laboratory.

Homing behaviors in the desert grassland scorpion, *Paruroctonus utahensis* (Williams 1968), are less understood. These nocturnal animals are ideal for homing studies because they are abundant, easy to obtain, and live in conditions that can be simulated in the laboratory. Scorpions have several sensory structures that may be used during homing. Their eyes, located laterally and medially on the prosoma, are sensitive to light levels typical of a clear nighttime sky (Fleissner & Fleissner 2001). The tip of the tail is also sensitive to light (Zwicky 1970; Rao & Rao 1973). They have tarsal taste hairs (Foelix & Schabronath 1983) and sensitive, mid-ventral organs called pectines (Cloudsley-Thompson 1955; Foelix & Müller-Vorholt 1983; Gaffin & Brownell 1997, 2001; Wolf 2008), which could be used to detect chemical and textural cues around their burrows. They also have mechano-sensory structures, including trichobothria (Hoffmann 1967; Messlinger 1987) on the pedipalps, which detect air currents and perhaps minute pressure changes around the burrow entrance (Gaffin 2011). Mechanosensory tarsal hairs could be used to detect previously made footsteps around the burrow.

Observations of scorpion homing behavior are typically conducted in the field, but studies are constrained by time of year and weather. In field studies, desert scorpions showed site fidelity after displacements of up to 8 m from their burrows, and straight-line return paths when < 50 cm from their burrows. Further, it is not uncommon for scorpions to be faithful to the same burrow for time spans ranging from months to years (Polis et al. 1986). We wanted to develop a lab-based assay to facilitate year-round observation and allow maximal control of potential sensory cues. Our objectives in this study were to induce scorpions to adopt artificial shelters in laboratory arenas, and to document their nocturnal surface activities in these arenas. We found that scorpions' pattern of shelter occupation was consistent with their normal field behavior in light and dark conditions. The scorpions moved consistently during nighttime hours and spent the majority of their out-of-shelter time close to the arena wall. Nevertheless, we noted occasional departures from the wall toward the arena center, sometimes directly into the mock burrow.

METHODS

Animal collection and care.—The subjects of this study were twelve female *Paruroctonus utahensis*. Eight were collected outside of Monahans, TX in the springs of 2010 and 2011, and four were collected near the Sevilleta Field Station, NM in the fall of 2012. They were each maintained in 3.8 L glass jars containing sand to a depth of approximately 2.5–5.0 cm and a piece of clay pot. Diet consisted of one cricket every 2 weeks. We moistened the sand weekly with approximately 5 mL of water to hydrate the scorpions. The laboratory was kept between 24–27 °C and 65–70% RH, with a light-dark phase of 0400–1800 h light and 1800–0400 dark.

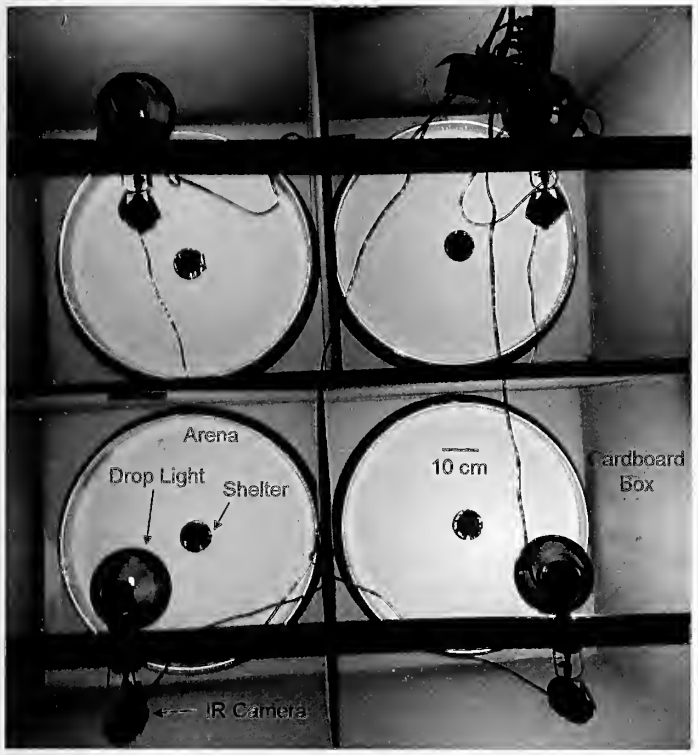

Figure 1.—Experimental apparatus. Scorpions are placed in circular arenas containing sand (2 cm deep) and an artificial shelter in the center. White clamp lights suspended above each arena simulate a normal light:dark cycle. Cameras are suspended above each arena to capture scorpion movements and emit IR light during the dark cycle for visibility. Cardboard boxes surround each arena to block light from adjacent arenas.

Arenas.—To approximate the sandy environments inhabited by the subjects, we set up four behavioral arenas, each consisting of a water heater drain pan (Camco, product #20813; 68.6 cm top diam., 65.4 cm base diam., 7.62 cm height). We covered the drainage hole (5-cm diam.) in the side of each pan with a strip of standard duct tape. The pans each contained 3.5 L of sand spread and leveled across the bottom (1 cm deep). We constructed shelters from two-part canning jar lids (Kerr home canning lid, 8.9 cm diam., 1.3 cm tall) from which three equidistant, 2.54 cm-wide slots were removed from the screw-on ring. The flat part of the lid was glued to the ring with Krazy Glue. Each shelter was placed on the sand in the middle of the arena. We placed the arenas in the bottoms of open-topped cardboard boxes (76.2 cm × 76.2 cm × 76.2 cm) to avoid shadow-cast on adjacent arenas. A droplight (Bayco 75-watt incandescent clamp light) was suspended 92.7 cm above the center of each arena. Each lamp contained an 8.6-watt bright white LED bulb (Ecosmart) (Figure 1). A dial timer switch controlled each light's operation on an approximated 14-hour on, 10-hour off cycle (lights off at 18:17, lights on at 04:16). We mounted one high-resolution infrared surveillance camera (Defender, 8-channel Smart Security DVR) next to each of the droplights (94 cm above arena). The camera videos were continuously captured on the DVR at four frames per second.

Site fidelity experiment.—Our experimental arenas were designed to supplement a previous experiment, where scorpions were shown to travel towards their burrow location in the laboratory (Bost & Gaffin 2004) after displacement. In the current study, our goal was to gather baseline activity patterns while also developing software to track their precise, long-term movements in artificial habitats.

We conducted two trials with four scorpions per trial. We placed a recently fed scorpion (Monahans collection) in each of the four arenas for 8 hours of acclimation. After acclimation, the subjects were recorded continuously for 7 days. Once the first trial ended, we mixed and leveled the sand to create a clean, unmarked surface before the second trial began.

We reviewed the footage and scored scorpion locations at 84 pre-selected times: seven during each light phase and five during each dark phase of the weeklong period (proportionate to the 14:10-hour light-dark cycle). We selected observation times using the RAND function in Microsoft Excel, eliminating times occurring within 1 hour of a previously selected time. Scorpions received a score of "1" if they were under the jar lid, and "0" if they were not. Each animal in the first trial received a total of 84 scores (49 light and 35 dark). We gave 83 scores in trial two (49 light and 34 dark) as a result of a temporary pause in videotaping during one of the dark observation times.

We compiled time-in-shelter percentages for all eight scorpions and applied a 2-tailed, paired t-test (our data were normally distributed). We then compared the percentages in light and dark conditions with the α-value set at 0.05.

Location mapping experiment.—To trace scorpion paths, we placed four *P. utahensis* (Sevilleta collection) in the arenas after cleaning and leveling the sand, and recorded them overnight. We analyzed footage during the first 7 hours of the first dark cycle (16:25–23:25) to correspond with the scorpions' normal peak activity period as seen in pilot studies using other *P. utahensis* scorpions. The DVR stored the video footage as several compressed files (each containing approximately one hour of footage). We converted these DVR compressed files (.264 format) into AVI files (AVI Generator 2.0) and finally to MP4 files (Converter Lite). We imported the MP4 files to iMovie (v. 9.0.9, Apple Inc.), split each file into two 30-minute segments, and increased speed to 2000% of initial footage. These files were then exported as .mov format (320 × 240 pixels) and imported into MATLAB (ver. R2012a, The MathWorks, Inc.).

Our MATLAB script used a frame-by-frame subtraction method to detect a scorpion's movement and plot its position in each video frame. We exported these coordinates to Excel. Occasional artifact coordinates were induced by poor video quality (such as from IR spotlighting and/or reduced resolution from converting video files through multiple formats). We categorized coordinates as artifacts if they met any of the following criteria: the point was outside the arena's radius; the point was more than 16 cm from the previous position; or the point was inconsistent with normal scorpion motion (e.g. a sharp 'spike' away from and then back towards a normal pathway). On other occasions, we identified artifacts by comparing suspiciously high position values with actual video footage.

After identifying and removing artifact coordinates, we created a macro that used the data to create a "movement density map" for each scorpion. The macro separated each

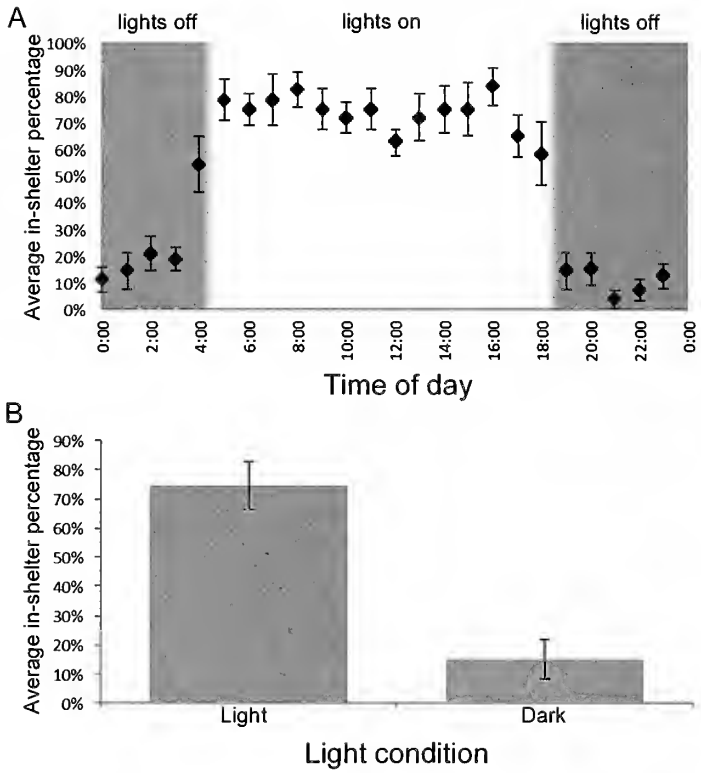

Figure 2.—A 10-day study indicates that scorpions show fidelity to artificial shelter sites in the lab. A) Average percentages of in-shelter observations at randomly selected times of the day. B) Average in-shelter percentages in "light" vs. "dark" conditions (mean ± SE).

arena into 0.648 em^2 sections (derived from a 101 × 101 cell matrix applied to the image) and plotted the amount of time (in seconds) that the scorpion spent moving in each section. These figures did not account for times where subjects were still. All data for one of the four scorpions were removed due to the presence of a live cricket in the arena that affected the scorpion's behavior.

RESULTS

Site fidelity.—The apparatus in the site fidelity experiment (Figure 1) was used to determine if scorpions would use artificial shelters similarly to natural burrows in the field. We first compared the averages of all the "in" scores by hour over the 7-day span (Figure 2A; for example, the data point with the x-value of "1:00" represents the average of all in-shelter percentages within all of the 1:00–1:59 time frames over the 7-day period). Fidelity was determined by comparing "in" values in light vs. dark conditions (Figure 2B). In light conditions, the eight scorpions averaged 74.5% ± 8.2% (mean ± SE) of the observed times inside their artificial shelter; in dark conditions, they averaged 15.0% ± 6.8% (mean ± SE). This pattern was significantly different from random at $P = 0.001$ ($t = 7.403$, d.f. = 7).

Location mapping.—We made long-term observations of scorpion surface activity as a baseline for future behavioral assays. Figure 3 shows the nocturnal movements of three scorpions, as well as their corresponding movement density maps. All three scorpions showed extensive wall-walking behavior interspersed with periodic wall departures and arced paths across the middle of the arena. The patterns of movement away from the walls were qualitatively different for the three scorpions. Scorpion 2 (Figure 3B) had the highest movement density in the arena center and made several off-wall excursions that retraced consistent elliptical paths. The off-wall exeursions of scorpions 1 and 3 appeared more random.

The density maps show the scorpions' positions during their periods of movement. During the 420-minute observation period, scorpion 1 was moving for a total of 118 min (28.1%; Fig. 3A), scorpion 2 moved for 129 min (30.7%; Fig. 3B), and scorpion 3 moved for 124 min (29.5%; Fig. 3C). The scorpions also varied in the percentage of total movement spent along the wall (all movement within 3 cm of the wall was considered "wall movement;" everything else was considered "center movement"). Scorpions 1, 2, and 3 spent 92%, 54%, and 86% of their movement time along the wall, respectively. Movements in the arena center sometimes included activity at the shelter openings. Scorpion 2 spent 17% of its arena center movements partially inside of the shelter, moving just enough for detection by our software.

DISCUSSION

The animals in this study treated their canning lid shelters with site fidelity analogous to home burrow inhabitation patterns in their native environment (Polis et al. 1985). Furthermore, cycling of the room lights to simulate a day-night pattern appears to induce outside-shelter activity patterns similar to those in the wild.

We developed MATLAB scripts to automatically track scorpion movement patterns. While this process greatly increases the speed and efficiency of gathering long-term movement data, several modifications could improve the assay. First, the duct tape covering the pre-cut hole in the drain pan influenced animal behavior; when reviewing video footage, we observed our subjects attempting to crawl on the duct tape-covered surfaces. Future studies should use plugs to seamlessly close the holes or use arenas without pre-cut holes. Second, video image quality should be increased to reduce tracking artifacts. A light dispersion filter could reduce spotlighting and make the IR coverage of the arena more uniform, allowing for more aecurate traeking during dark hours. Limitations imposed by the resolution of the surveillance system (eoupled with additional losses imposed by file conversion) could be overcome by using a higher resolution system, but that would generate additional data storage demands. If we could directly extract and store movement data without saving the extraneous arena information, this issue could be mitigated.

This in-lab site fidelity assay provides a useful tool for addressing questions of scorpion homing because it is not limited by challenges imposed by field conditions. Furthermore, automated tracking of scorpion movements allows for more efficient analysis and concurrent assessment of multiple subjects in similar environmental conditions. We plan to use this assay to systematically deduce the primary sensory cues used by animals as they return to their home shelters. Since scorpions more often returned to their shelters in lit conditions than in dark, it may be useful to displace scorpions from their shelters with the lights on, which would presumably prompt an immediate return path recordable by our software. An

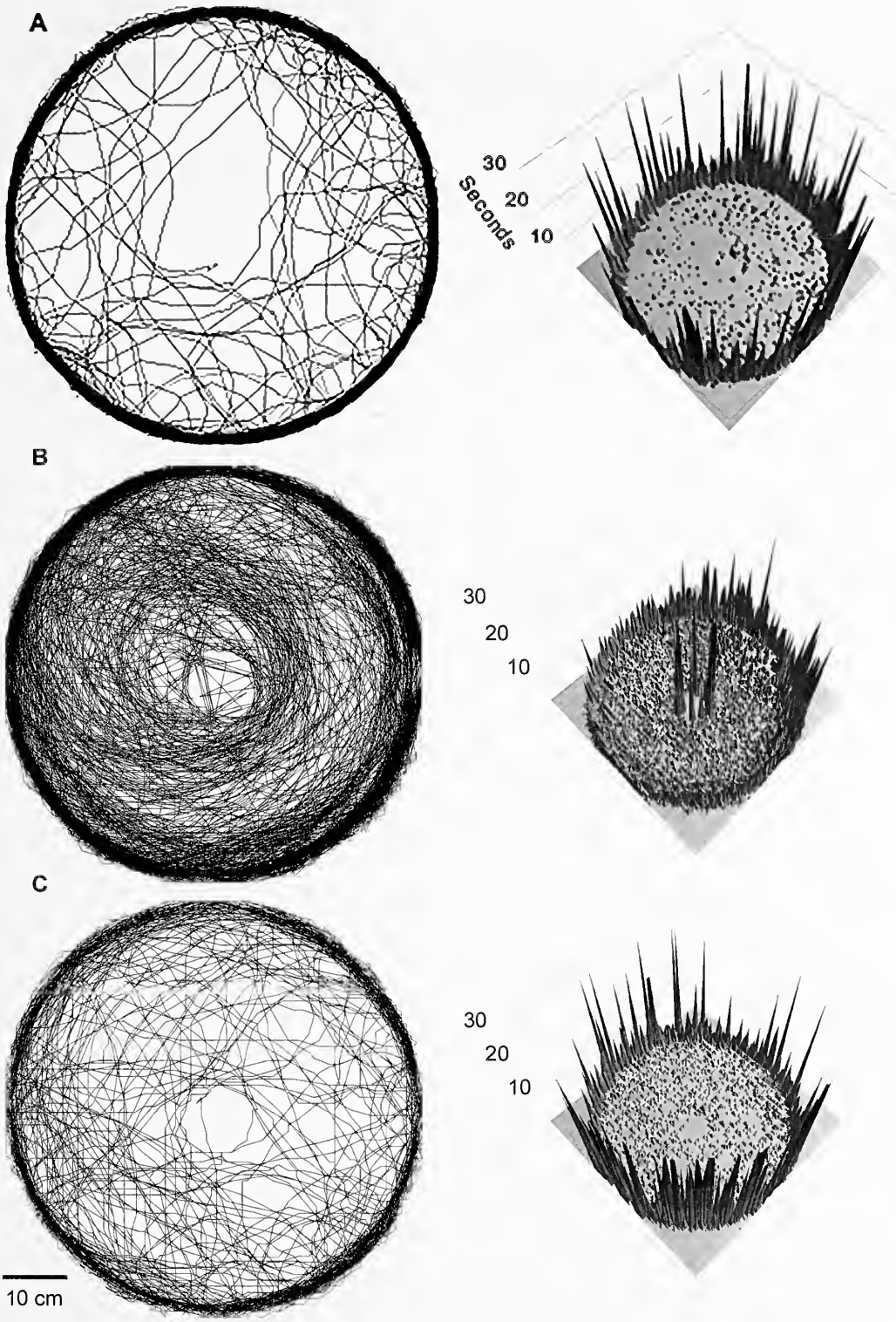

Figure 3.—Activity of three scorpions over a 7-hour dark period. A, B, and C represent individual scorpion data. Left panels: Path data collected from day 1 during the first 7 hours of darkness. The circle of relative absence in the center of each plot is the location of the shelter. In the second plot's center, the triangular plot pattern's vertices connect the three shelter entrances. Right panels: Z-axis values represent plot density measured in seconds of movement per measured unit area (0.648 cm^2).

alternative would be to change the on/off light cycles to last a much shorter period of time, which may induce homing behavior more often.

Scorpions might use several strategies to find their burrows. For example, because scorpion eyes are sensitive to very low light levels (Fleissner & Fleissner 2001), star patterns might be used as a homing reference. Even in our indoor arenas, the animals could have been using other sources of light within the room. However, the animals were filmed under IR light (which they cannot see; Camp & Gaffin 1999) and the cardboard

boxes blocked direct light from other sources (such as the LEDs on the video recorder).

Scorpions also have chemo- and mechanosensory sensilla on their tarsi and pectines that could be used to track self-made chemical deposits and/or footstep patterns back to their home burrows. Disrupting the sand around the artificial shelter while the animal is at the arena wall should help reveal the importance of these cues.

In previous studies, we have filmed animals under IR light in their native habitats as they made excursions from and to their home burrows (Gaffin 2011). Animals would often wait at their burrow threshold until induced to emerge by the vibrations caused by nearby moving arthropods. We also found we could coax animals to follow small wads of paper pulled past their burrows with thread. In another study, we coaxed an animal onto a rigid platform buried just beneath the sand surface. When we displaced the platform several centimeters, the scorpion's first movements were to a point where its burrow would have been had it not been displaced (Gaffin et al. 2012). This suggests that the animals may use a path integration mechanism for approximating the angle and distance to their burrow. Using such coaxing and displacement strategies in our in-lab assay should provide additional insights into the use of path integration. If true, this sets up the possibility that the animals might track familiar sensory cues experienced during the initial home foray established by path integration (Baddeley et al. 2012).

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Substrate selection and spatial segregation by two congeneric species of *Eustala* (Araneae: Araneidae) in southeastern Brazil

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Abstract. Habitat and microhabitat selection by spiders are influenced by abiotic and biotic factors, including vegetation structure, natural enemies, and prey availability. Some species are highly dependent on particular conditions, such as the presence of substrates where they remain camouflaged, constantly humid sites or the occurrence of plants bearing glandular trichomes. Others are distributed in areas that include a wide range of physical conditions and interact with several types of prey, predators and competitors. In the present study, we evaluated spatial distribution and substrate selection of two sympatric congeneric species with distinct body shapes and colors, *Eustala taquara* (Keyserling 1892) and *E. sagana* (Keyserling 1893), in an area of Atlantic Forest in southeastern Brazil. We focused on the following factors regarding habitat selection: i) distance from the border (forest edge or interior); ii) altitudinal distribution, ranging from 740 to 1294 m; iii) web height above ground level; and iv) plant species used for web attachment. All individuals of both species were located at the forest edge, especially on dry branches. However, they occurred preferentially in different host plants and altitudes. *Eustala taquara* individuals were strongly associated with *Conyza bonariensis*, and *E. sagana* with *Hyptis suaveolens* and *C. sumatrensis*. Dry branch preferences might be important to reduce species conspicuousness to visually oriented predators, such as birds and wasps. Spatial segregation between closely related species possibly minimizes interference interactions, such as competition for particular sites or prey items.

Keywords: Orb weaver spider, coexistence, altitudinal gradient, habitat selection, web building

Microhabitat selection during web construction has important implications for web-building spiders, affecting the frequency of prey interception (Heiling 1999; Herberstein & Fleisch 2003; Heiling et al. 2006), prey species availability (Herberstein 1997), interspecific competition and/or frequency of agonistic interactions with other spiders or organisms (Herberstein 1998; Nahas et al. 2012; Purcell et al. 2012). Microhabitat selection could also expose spiders to inappropriate environmental conditions (Gillespie 1987; Gonzaga et al. 2006), to predators (Gonzaga & Vasconcellos-Neto 2005) and to other sources of mortality, such as infections by fungi (Gonzaga et al. 2006).

The first requirements for web establishment are associated with site structural conditions, such as spatial distribution of branches used in web attachment, and an open space large enough to facilitate construction of the web (Stevenson & Dindal 1982; Rao & Poyyamoli 2001). Spiders, however, often have more specific criteria, and can use several cues to assess site quality before initiating web settlement. For example, Schuck-Paim & Alonso (2001) showed that *Nephilengys cruentata* (Fabricius 1775) (Nephilidae) used signs of previous conspecific presence to evaluate habitat quality. Conspecific silk inside the enclosures employed in the experiments increased settlement likelihood by *N. cruentata* individuals.

Plant characteristics can also be integral in determining initial settlement, establishment success, and permanence of several spider species (Romero & Vasconcellos-Neto 2005). Selectivity for particular host plants has been previously described for many spider species (Romero & Vasconcellos-Neto 2005, 2011; Romero et al. 2008; Hesselberg & Triana 2010). Spiders select plants with specific attributes, e.g., presence of glandular trichomes, adequate branch and leaf densities, pollination by potential prey, color pattern, and structural composition that can serve as suitable shelter, which consequently increases each species potential to improve prey capture success, and reduces the likelihood of capture by natural enemies.

The genus *Eustala* Simon 1895 (Araneidae), restricted to the Americas, is currently composed of 90 species (Platnick 2014). Individuals of several *Eustala* species are often distributed throughout shrub and tree vegetation (Poeta et al. 2010), and are the primary prey species of several hunting wasps (Gonzaga & Vasconcellos-Neto 2005; Buschini et al. 2008). In Serra do Japi, an area of Atlantic Forest situated in southeastern Brazil, three species have been previously collected (Indicatti et al. 2012): *Eustala perfida* Mello-Leitão 1947, which typically occurs in shaded areas of forest interior and constructs elongated orbs close to concavities on tree trunks (Messas et al. 2014); *E. sagana* (Keyserling 1893); and *E. taquara* (Keyserling 1892). Habitat selection and spatial distribution of *E. perfida* was previously studied by Messas et al. (2014), but there is no information available on the remaining species. We hypothesized that *E. sagana* and *E. taquara*, two species with distinct body shapes and colors, would exhibit differences in daytime resting substrates. In addition, we evaluated spatial segregation in these species by analyzing habitat selection based on vegetation height and altitude within the distribution area.

METHODS

Study site.—We conducted the present work in the Serra do Japi (23° 11′ S, 46° 52′ W), a forest reserve located near the city of Jundiaí, São Paulo state, Brazil. The climate is seasonal, with average monthly temperatures from 13.5° C

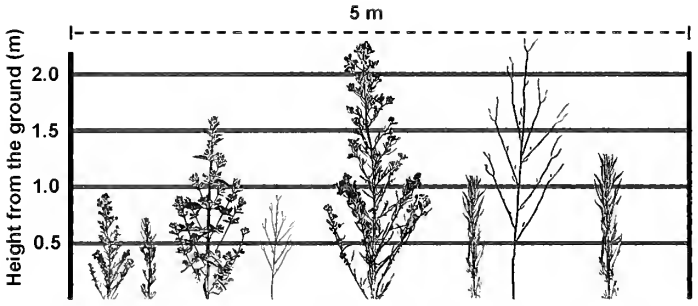

Figure 1.—Schematic drawing of the methodology employed to analyze the frequency of available sites (vegetation height) in the Serra do Japi, Jundiaí-SP. Four 5-m lines, separated vertically every 0.5 m, were arranged in the vegetation; all plants touched by the lines were included.

in July to 20.3° C in January. The driest period is from June to September, and the rainy season is from December to February (Pinto 1992). The local vegetation is characterized by a semi-deciduous rainforest with canopy height varying between 10–15 m. The higher mountain regions support a semideciduous forest composed of thinner trees (Leitão-Filho 1992). The altitude in the Serra do Japi ranges from 740–1294 m.

Spatial distribution.—All sampling was conducted in March 2011. We examined the distance from the border (forest edge or interior) and the altitudinal distribution of both species. *Eustala taquara* and *E. sagana* altitudinal distribution and edge effects were investigated by sampling forest edge and interior in four altitudinal ranges: 750–800 m, 1000 m, 1250 m, and 1294 m. In this study, we considered the forest edge as a

Table 1.—List of available plant species present on sample plots and frequency and number of individuals of *Eustala sagana* found occupying plants along the edge of the forest in Serra do Japi, Jundiaí – SP, Brazil. The data were collected at an altitude of 800 m during May and July 2011.

		Plants		*E. sagana*	
Family	Species	*n*	%	*n*	%
Anacardiaceae	*Lithraea molleoides*	1	0.15	0	0
Asclepidadaceae	*Asclepias curassavica* L.	1	0.15	0	0
Asteraceae	*Cyrtocymura scorpioides* (Lam.) H. Rob.	125	18.71	0	0
	Eupatorium sp.	32	4.79	0	0
	Vernonanthura ferruginea (Less.) H. Rob.	31	4.64	**9**	8.18
	Vernonanthura phosphorica (Vell.) H. Rob.	17	2.54	0	0
	Trichogoniopsis adenantha (DC.) R.M.King & H.Rob	12	1.8	**2**	1.82
	Baccharis dracunculifolia DC.	4	0.6	**1**	0.91
	Bidens brasiliensis Sherff	4	0.6	**3**	2.73
	Calea pinnatifida (R. Br.) Less.	8	1.2	0	0
	Conyza bonariensis (L.) Cronq.	5	0.75	0	0
	Conyza sumatrensis (Retz.) E. Walker	15	2.25	**35**	31.82
	Erechtites valerianifolius (Link ex Spreng.) DC.	0	0	**3**	2.73
	Mikania sp.	5	0.75	0	0
	sp.1	66	9.88	0	0
	sp.2	4	0.6	0	0
	Acanthospermum hispidum	0	0	**1**	0.91
	Tagetes minuta L.	0	0	**1**	0.91
Bignoniaceae	*Pirostegia venusta* (Ker-Gawl) Miers	2	0.3	0	0
Cecropiaceae	*Cecropia* sp.	2	0.3	0	0
Euphorbiaceae	*Croton floribundus* Spreng	1	0.15	0	0
Fabaceae	sp.1	6	0.9	0	0
Lamiaceae	*Hyptis suaveolens* L.	47	7.04	**26**	23.64
	Hyptis sp.1	13	1.95	**3**	2.73
Malvaceae	*Triumfetta semitriloba* Jacq.	36	5.39	**1**	0.91
	sp. 1	0	0	**3**	2.73
	sp. 2	1	0.15	0	0
Melastomataceae	sp. 1	9	1.35	0	0
Piperaceae	*Piper* sp.	2	0.3	0	0
Poaceae	*Brachiaria decumbens* Stapf.	168	25.15	0	0
	Panicum maximum Jacq.	21	3.14	0	0
	Grass	0	0	**9**	8.18
Pteridophytes		5	0.75	0	0
Rosaceae	*Rubus rosifolius* BP. MS.	4	0.6	0	0
Rubiaceae	sp.1	2	0.3	0	0
Solanaceae	*Solanum concinnum* Schott ex Sendtn.	10	1.5	0	0
	Solanum variabile Mart.	3	0.45	0	0
	Sessea brasiliensis Toledo	2	0.3	0	0
Verbenaceae	*Lantana camara* L.	4	0.6	0	0
Others	Rare species	0	0	**10**	9.09
	Dry branches	0	0	**3**	2.73
Total		0668	100	110	100

Table 2.—List of available plant species present on sample plots, and frequency and number of individuals of *Eustala taquara* found occupying plants along the edge of the forest in Serra do Japi, Jundiaí – SP, Brazil. The data were collected at an altitude of 1000 m in March 2011.

Family	Species	Plants		*E. taquara*	
		n	%	*n*	%
Asteraceae	*Ageratum conyzoides* L.	1	0.54	0	0.00
	Ambrosia artemisiifolia L.	11	5.95	4	2.65
	Bidens brasiliensis Sherff	2	1.08	3	1.99
	Calea pinnatifida (R. Br.) Less.	2	1.08	0	0.00
	Conyza bonariensis (L.) Cronq.	14	7.57	103	68.21
	Cyrtocymura scorpioides (Lam.) H. Rob.	4	2.16	0	0.00
	Erechtites valerianifolius (Link ex Spreng.) DC.	1	0.54	2	1.32
	Eupatorium sp.	2	1.08	0	0.00
	Trichogoniopsis adenantha (DC.) R.M.King & H.Rob	0	0.00	2	1.32
Euphorbiaceae	*Croton floribundus* Spreng	8	4.32	0	0.00
	Croton urucurana Baill	5	2.70	0	0.00
Fabaceae	*Acacia plumosa* Lowe	1	0.54	0	0
	Desmodium discolor Vogel	0	0.00	3	1.99
Lamiaceae	*Hyptis suaveolens* L.	49	26.49	10	6.62
Malvaceae	sp.1	24	12.97	0	0.00
Melastomataceae	*Miconia* sp.	2	1.08	0	0.00
	sp.1	2	1.08	0	0.00
Myrtaceae	sp.1	0	0.00	2	1.32
Piperaceae	sp.1	8	4.32	0	0.00
Poaceae	Grass – sp.1	2	1.08	0	0.00
	Unidentified	5	2.70	0	0.00
Rosaceae	*Rubus rosifolius* BP. MS.	2	1.08	0	0.00
Rubiaceae	sp.1	3	1.62	5	3.31
Solanaceae	*Solanum concinnum* Schott ex Sendtn	2	1.08	0	0.00
	Solanum variabile Mart	2	1.08	0	0.00
	Sesseia brasiliensis Toledo	2	1.08	0	0.00
Verbenaceae	*Lantana camara* L.	2	1.08	0	0.00
Others	Pteridophytes	13	7.03	0	0.00
	Eudicotyledons	1	0.54	2	1.32
	Dry branches	15	8.11	13	8.61
Total		185	100	151	100

zone of 2 m from the road margin towards the inner forest. This area is covered by herbaceous plants and shrubs located between the road and arboreal vegetation. For each site, individuals from 20 × 2 m plots (n = 15) were sampled at the forest edge, and individuals from 10 × 10 m plots (n = 12) were sampled within the forest. Shape and size of the plots in the forest edge were restricted by the area available under the influence of the wide open trails and roads. We sprayed water in vegetation to locate webs during the searching procedure.

We compared the number of *E. taquara* and *E. sagana* individuals at distinct altitudes using a non-parametric Kruskal-Wallis test, as initial analyses showed the data distribution lacked homogeneity of variances. Only plots located at the edge of trails were considered as replicates because both species were absent from plots located in the forest interior. Post-hoc Dunn's multiple comparison tests (Dunn 1964) were applied to compare the abundances between altitudes.

Substrate selection.—We searched for spiders along the forest edge in order to obtain the observed values of web height from ground level, green and dead substrate and plant species occupied. Samples were collected where each species was more abundant, at altitudes of 800 m for *E. sagana* during May and July 2011, and at 1000 m for *E. taquara* in March 2011.

We evaluated whether *E. sagana* and *E. taquara* exhibited specific height preferences (above ground level) by establishing 5 m transects (n = 8) in the two altitudes with higher occurrence of each species. We extended lines at four different heights (0.5 m; 1.0 m; 1.5 m; and 2.0 m), and counted the number of times each line intersected vegetation (Fig. 1). The total number of intersections was used to establish the availability of sites at different sample heights. We used the number of intersections at each height as the expected frequency for spider occupation. These frequencies were compared with the frequencies of observed web heights used by *E. taquara* and *E. sagana* using a G test.

Substrate selection for web construction in *E. taquara* and *E. sagana* was investigated in 10 × 2 m plots (n = 8) also on the forest edge. Within these plots we calculated the number of sites available with green and dry branches (open spaces wide enough to allow web construction) and the proportion of each plant species (Table 1 and 2). We compared the available number of green and dead branches with the number of these kinds of branches occupied by *Eustala* spiders. We also compared the relative abundance of plant species in plots (expected frequencies), at the altitude with highest abundance of each spider species, with the relative abundance of plants used as web-building sites along the forest edge (observed frequencies) applying G test.

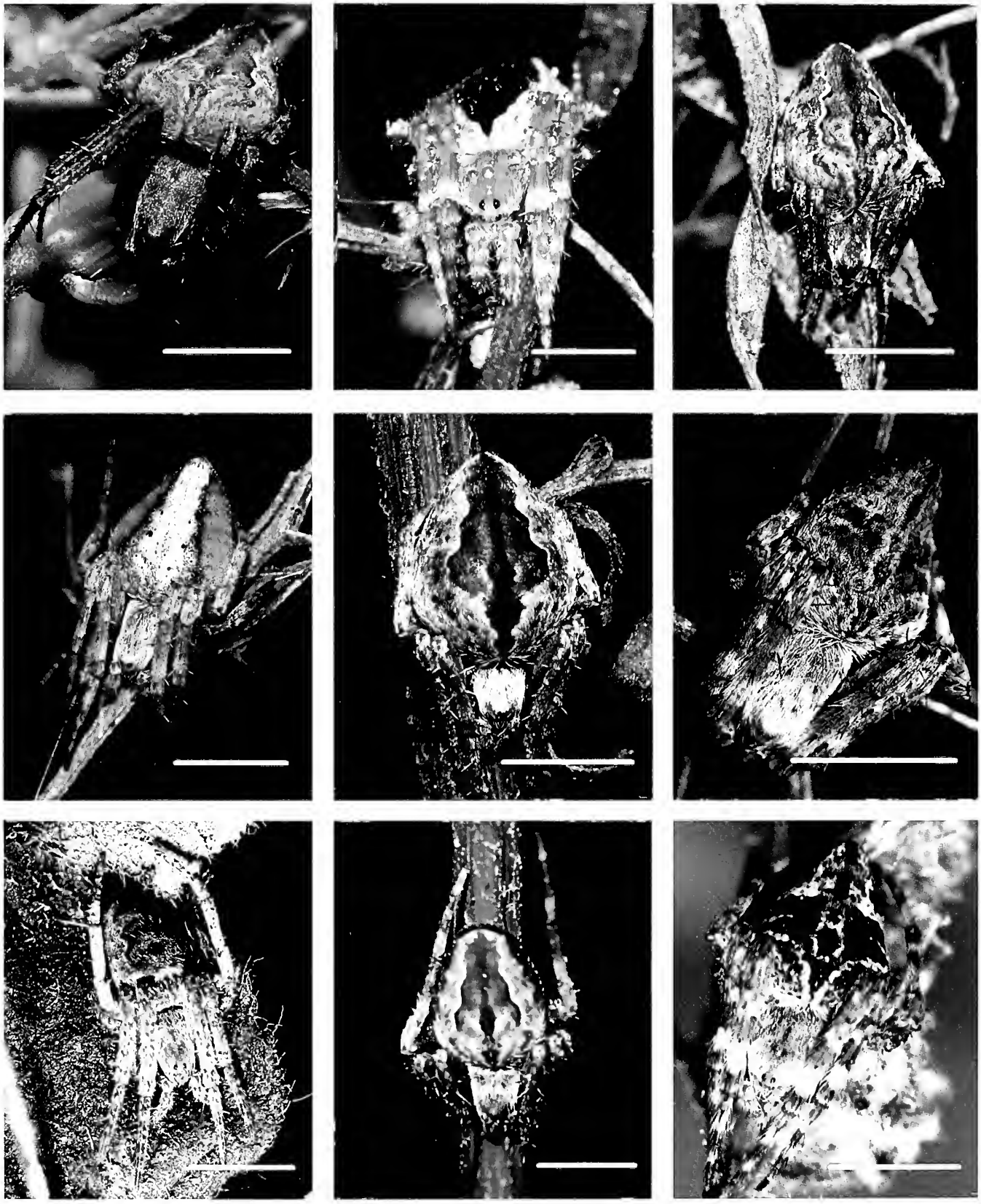

Figure 2.—*Eustala taquara* specimens resting on dead (dry) vegetation, with coloration pattern details. Scale bar: 0.5 cm.

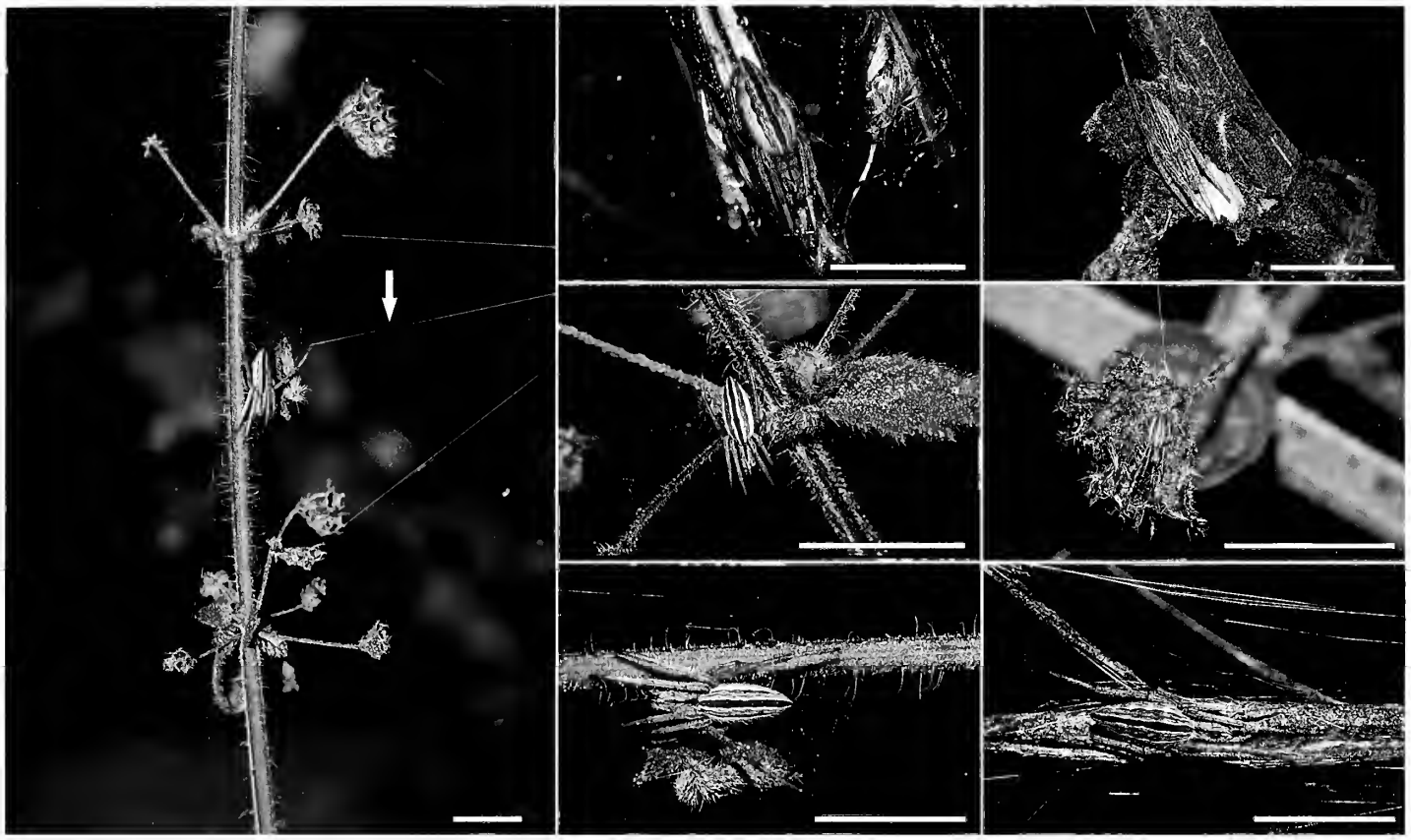

Figure 3.—*Eustala sagana* specimens resting on dry vegetation. Detail of a free sector of the web which is lacking capture spirals. The arrow indicates the signal thread that connects the spider to the web center. Scale bar: 1.0 cm.

RESULTS

Results showed both *Eustala* species did not build shelters, but were instead found in shrub branches, which provided attachment points for web construction. Spiders remained in contact with branches, holding a web thread connected to the hub of the orb web. The spider color patterns were very similar to the substrate patterns used as resting positions (based on the human visual system) (Figs. 2, 3).

Spatial distribution.—All *Eustala taquara* (n = 97) and *E. sagana* (n = 46) individuals were located at the forest edge. Both *Eustala* species were sampled at all altitudes of Serra do Japi, but with different density patterns. A significantly higher abundance of *E. sagana* was detected in the lower Serra do Japi area, between 750–850 m (91.3% of all sampled individuals) (Kruskal-Wallis: H = 23.08, DF = 3, P <0.0001; *Dunn* $Z_{800m \times 1000m}$ = 3.73, P = 0.001; $Z_{800m \times 1250m}$ = 3.99, P = 0.0004; $Z_{800m \times 1294m}$ = 3.99, P = 0.0004; $Z_{1000m \times 1250m}$ = 0.27, P = 1.0; $Z_{1000m \times 1294m}$ = 0.27, P = 1.0; $Z_{1250m \times 1294m}$ = 0.00, P = 1.0; Fig. 4A). We found 20.6% of *Eustala taquara* individuals at 750 m; 52.6% at 1000 m; 16.5% at 1250 m; and 10.3% at 1294 m of altitude, with a significantly higher abundance at the intermediate zone (1000 m) (Kruskal-Wallis: H = 19.24; DF = 3; P <0.001; *Dunn* $Z_{800m \times 1000m}$= 3.27, P = 0.006; $Z_{800m \times 1250m}$ = 0.005, P = 1.0; $Z_{800m \times 1294m}$ = 0.66, P = 1.0; $Z_{1000m \times 1250m}$ = 3.26, P = 0.007; $Z_{1000m \times 1294m}$ = 3.93, P = 0.0005; $Z_{1250m \times 1294m}$ = 0.66, P = 1.0; Fig. 4B).

Substrate selection.—Lines intersected vegetation more frequently at the 0.5 m level above the ground, and decreased gradually as heights increased to 2.0 m. However, results showed *E. sagana* occurrence was highest at 1.0 m (30.6%) and 1.5 m (46.4%) above ground level (G = 115.29; P <0.0001; n = 117 sites; n = 106 spiders; Fig. 5A). *Eustala taquara* frequencies were significantly higher at 1.0 m (39.6%) and 1.5 m (44.3%) compared to the expected frequencies considering the available sites (G = 97.80; P <0.0001; N sites = 188; N spiders = 108; Fig. 5B).

Almost all *E. sagana* individuals (96.9%) were observed using dead vegetation branches (Fig. 6A), indicating a preferential use for these sites (G = 197.16; P <0.0001; N sites = 596; N spiders = 96). *Eustala taquara* exhibited the same pattern, with most individuals (96.6%) also sampled on dead branches (Fig. 6B), indicating preferred use of these sites (G = 167.27; P <0.0001; N sites = 315; N spiders = 146).

Host plants.—*Eustala sagana* occurred preferentially on *Hyptis suaveolens* (L.) Poit (Lamiaceae), *Conyza sumatrensis* (Retz.) E. Walker (Asteraceae), and *Vernonanthura ferruginea* (Less.) H. Rob. (Asteraceae) (G = 278.5; P <0.0001; Fig. 7A; Table 1), while *E. taquara* occurred mainly on *C. bonariensis* (L.) Cronquist (G = 107.70; P <0.0001, Fig. 7B; Table 2).

DISCUSSION

Both species exhibited similarities in habitat selection, and showed preferential use and restriction to forest borders, dead vegetation branches, and heights between 1.0 and 1.5 m above ground level. In contrast, results showed each species was distributed preferentially at a specific altitude, and on distinct plant species. These results are consistent with our hypothesis that the species are spatially segregated.

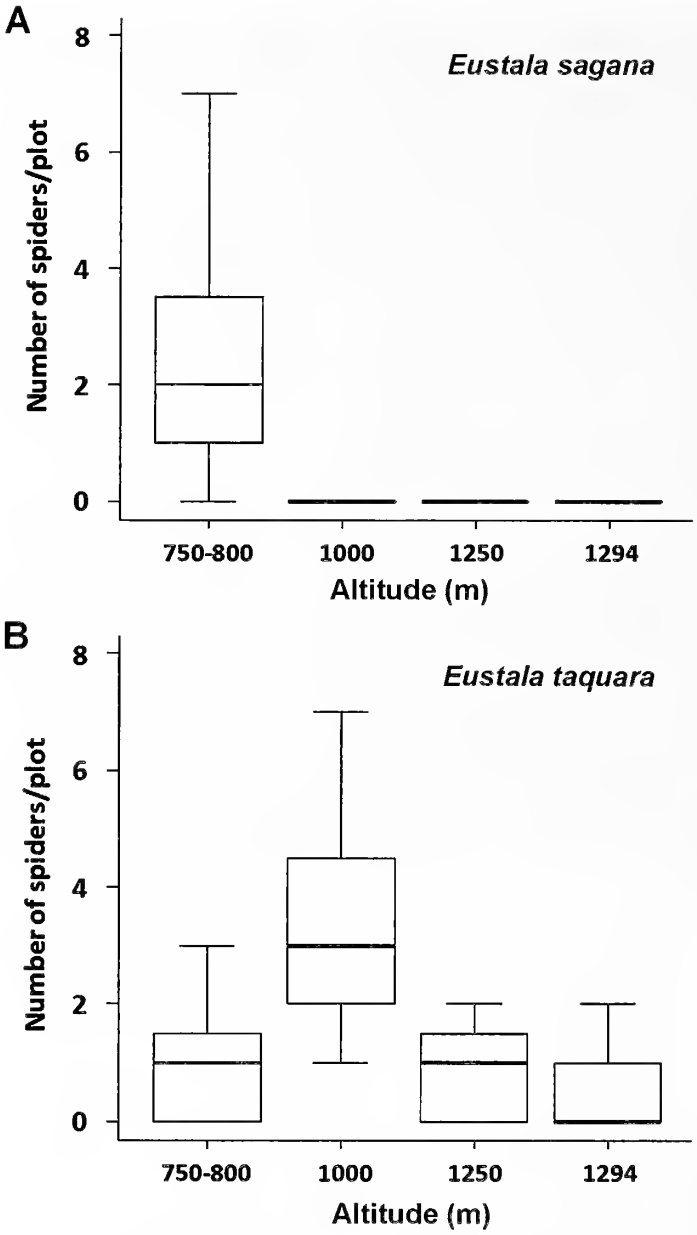

Figure 4.—*Eustala sagana* and *E. taquara* densities at different altitudes of the Serra do Japi, Jundiaí-SP. Box plots show median and interquartiles. Whiskers represent 1.5 × interquartile ranges.

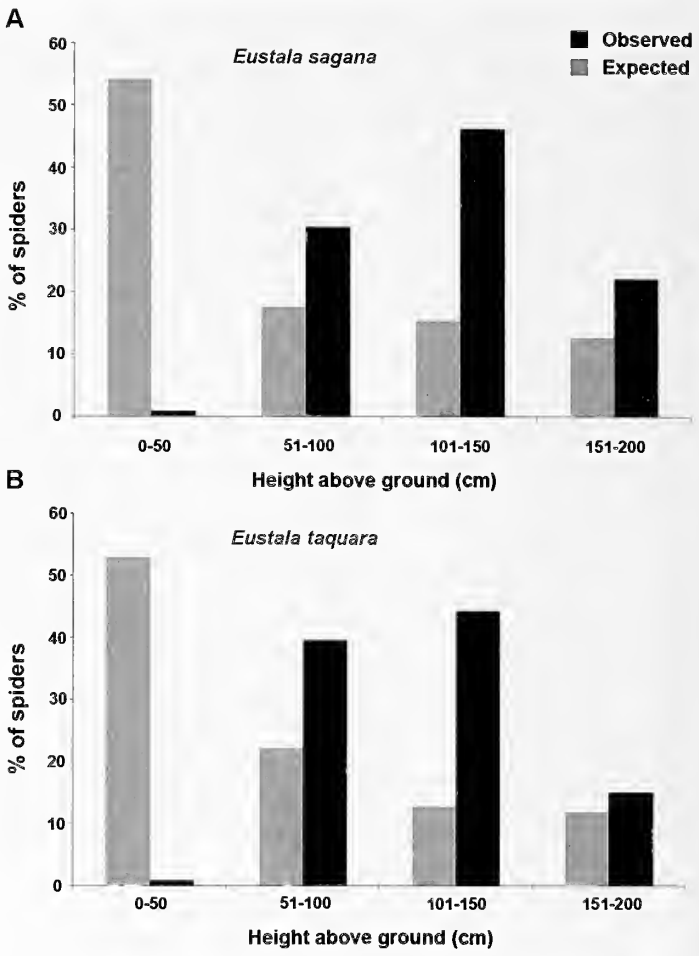

Figure 5.—Available (vegetation height) and observed sites used by *E. sagana* and *E. taquara* in Serra do Japi, Jundiaí-SP.

Eustala taquara occurred at a higher abundance at 1000 m, while *E. sagana* exhibited higher abundance at lower altitudes (750–850 m). Preferences regarding altitude might be a response to requirements for specific abiotic conditions (temperature, humidity, exposure to solar radiation), or to biotic criteria, including obtainable prey, exposure to predators, or availability of suitable web construction sites (Turnbull 1973; Brown 1981; Janetos 1986; Lubin et al. 1991; Marshall & Rypstra 1999). Similarly, Purcell & Avilés (2007) observed that altitude is an important factor affecting *Anelosimus* Simon 1891 (Theridiidae) species distribution and colony size (within social species of this genus) in Ecuador. The same authors suggested the influence of altitude occurred primarily in response to biotic factors, such as prey size and predator pressure; large insects were more common in lowland rain forests, and predator pressures were reduced at higher altitudes. In addition, it is possible that spatial segregation between closely related species is a response to competition (but see Wise (1993) for evidence against competition in spiders). In a future investigations we intend to examine prey availability (types and sizes) for each species, web architecture variation, and possible variation in abiotic conditions along the altitudinal gradient to evaluate the causes of spatial segregation. It is also important to evaluate possible differences in the abundance of plant species along the gradient as a component of habitat selection.

Eustala taquara individuals were associated with herbaceous plants and shrubs that occurred on the forest edge, primarily *C. bonariensis*. This species apparently avoids constructing webs in *H. suaveolens*, which is preferentially used by *E. sagana*. Respective associations of *E. illicita* (O. Pickard-Cambridge 1889) and *E. oblonga* Chickering 1955 with *Acacia collinsii* Safford and *A. melanoceras* Beurl. have previously been characterized (Chickering 1955; Hesselberg & Triana 2010), suggesting habitat selection by some *Eustala* species. Despite the association of *E. illicita* with *A. collinsii*, Hesselberg & Triana (2010) also observed these spiders occupying dead vegetation along the study site, and concluded the relationships between *Eustala* and vegetation species are not obligatory.

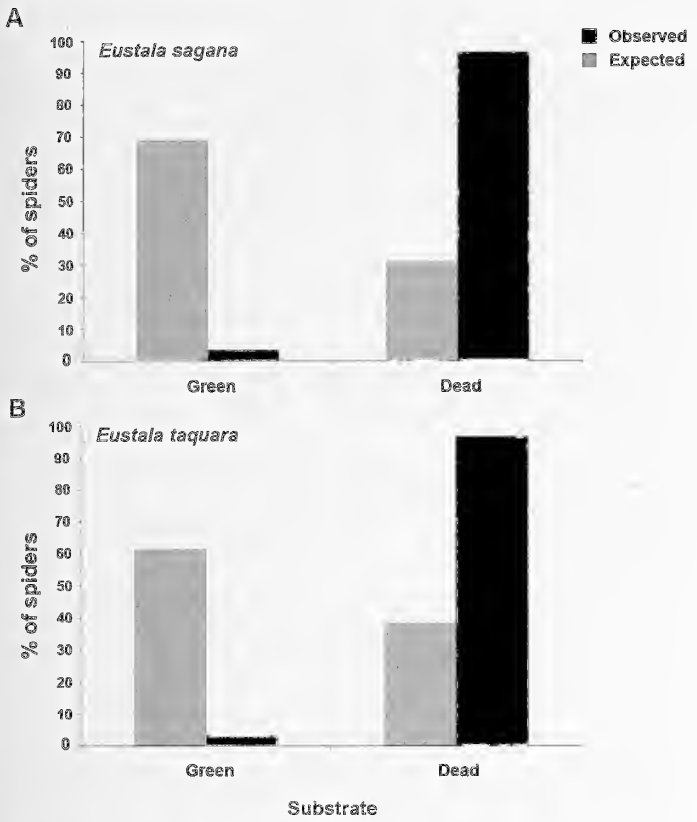

Figure 6.—Available green and dry (dead) vegetation sites and sites used by *Eustala sagana* and *E. taquara* in Serra do Japi, Jundiaí-SP.

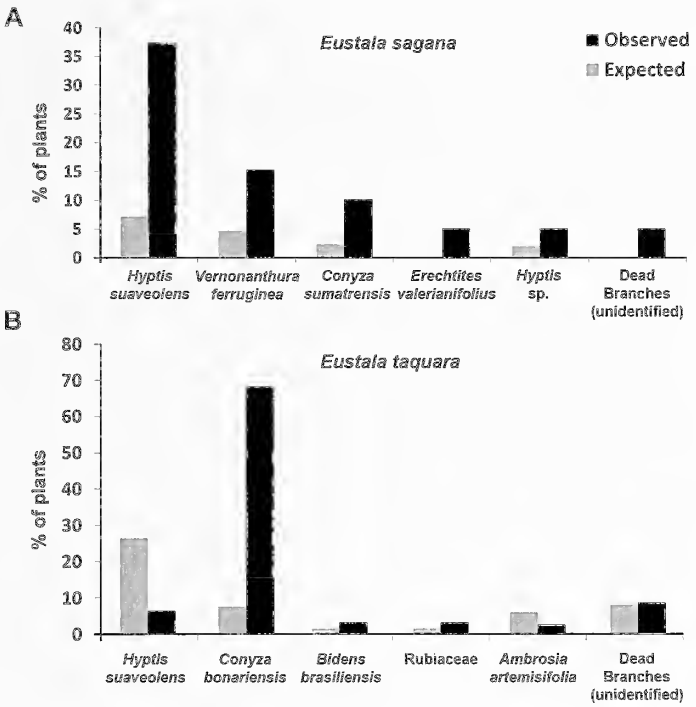

Figure 7.—Available plant species and plants occupied by *Eustala sagana* and *E. taquara* in Serra do Japi, Jundiaí-SP.

The selection of specific sites for web construction by *E. taquara* and *E. sagana* may be important to potentially reduce their conspicuousness and, consequently, exposure to visually oriented predators (such as birds and hunting wasps). We found that individuals of both species were apparently cryptic when resting in dry vegetation during the day. We have no information on their predators in Serra do Japi, but several empirical studies have demonstrated that *Eustala* species are hunted by *Trypoxylon* Latreille 1796 (Crabronidae) wasps (Rehnberg 1987; Gonzaga & Vasconcellos-Neto 2005; Buschini et al. 2008, 2010). *Eustala* is the most common prey found in nests of *Trypoxylon albonigrum* Richards 1934, for example, in another Atlantic forest area in São Paulo state (Gonzaga & Vasconcellos-Neto 2005; Araújo & Gonzaga 2007). It is important now to evaluate the ability of birds and wasps to perceive the chromatic and achromatic contrasts between the spiders and the substrates selected for web construction.

To conclude, this study showed that two congeneric sympatric species of *Eustala* present some similarities regarding habitat characteristics, such as preferential distribution close to the forest borders and occurrence on dead vegetation branches. However, there were also important differences in the two species' distributions. Their spatial segregation at distinct altitudes might reduce interference interactions between species. Future studies on prey preferences and experimental procedures manipulating the density of each species are essential to understanding the possible impact of each species on the abundance and habitat selection of the other.

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Activity patterns of a synanthropic population of the brown recluse spider, *Loxosceles reclusa* (Araneae: Sicariidae), with observations on feeding and mating

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Abstract. I recorded diel and seasonal activity patterns and behavior of the brown recluse spider, *Loxosceles reclusa* Gertsch & Mulaik 1940 in a free-ranging synanthropic population in northwestern Illinois. Recluse spiders are sit-and-wait predators that spend 85–90% of their nocturnal activity sitting motionless on a small network of silk they use for prey detection. Time not spent waiting for prey is typically occupied maintaining the web by laying down new strands of silk. Feeding and sexual behavior constitute a minute, but critical, portion of daily activity. Recluses were more active at night, but some were active during the day, especially in darker areas of the garage. Activity was relatively constant during the nocturnal hours. Recluse spiders became active in early to mid-May and ceased in mid-October. Beyond this, there was no consistent pattern observed in activity through these months. Sexual encounters were typically brief and similar to behavior reported in prior lab studies. Agonism was rare, but intraspecific predation was the most significant contributor to observed mortality. The most commonly captured prey in this population were spiders (Araneae, 25%), beetles (Coleoptera, 21%) wood lice (Isopoda, 15%), and crickets (Orthoptera, 13%). Recluse spiders were never observed actively searching for prey, live or dead. More than 80% of dead prey that were offered experimentally were not scavenged. Brown recluse spiders are not active scavengers; they are sit-and-wait predators that will take advantage of dead prey they happen to encounter during other activities.

Keywords: Diel, seasonal, predation, scavenging, phenology, behavior

Despite their notoriety, the behavior of brown recluse spiders (*Loxosceles reclusa* Gertsch and Mulaik 1940) has not been well-studied in natural or synanthropic settings. While other species of *Loxosceles* have been studied in the field to a limited degree (e.g., Richman 1973; Stropa 2007; Fischer & Vasconcellos-Neto 2005), to date, behavioral studies of *L. reclusa* have been conducted on captive laboratory populations (Hite et al. 1966; Sandidge 2003; Sandidge & Hopwood 2005; Parks et al. 2006; Cramer 2008; Cramer & Maywright 2008) rather than on recluse spiders in natural or synanthropic populations associated with human habitation. In their seminal study of behavior and basic biology, Hite et al. (1966) stated "There was not a single paper dealing with the biology of the brown recluse" prior to their study. Hite et al. (1966) were the first to examine feeding, mating, reproduction and development, and temperature tolerance among other aspects of this medically important species, but did so entirely in a laboratory setting.

Since then, a handful of additional laboratory studies have added to our basic knowledge of brown recluse spiders. Horner & Stewart (1967) expanded on reproductive biology (growth, mortality, egg sac number, etc.) and longevity, and Elzinga (1977) followed with more on longevity showing that recluses not exposed to extreme heat could survive easily for four or more years in the lab. Eskafi et al. (1977) found that recluse spiders had extremely low rates of water loss, the third lowest of any arthropod tested to that point. Recluse spiders apparently use metabolic water because their survival was more dependent on recent feeding than ambient humidity or the percent water content of the body, which was constant at death. Cramer & Maywright (2008) proposed cold temperature as a limiting factor in naturally occurring populations of recluse spiders in Illinois, using lab temperature tolerance tests and historical winter minimum temperatures to extrapolate a hypothetical northern extent of their range that matched fairly well with their known distribution.

Sandidge (2003) renewed interest in recluse spider foraging behavior by finding that they would often eat or even prefer dead over live prey offered in a laboratory in small enclosures. However, in similar laboratory tests, Cramer (2008) found that recluse spiders preferred live prey over dead, with level of food stress, prey decay and size of live prey as other important variables influencing prey choice. Additionally, Vetter (2011a) showed that 28 of 29 non-*Loxosceles* spiders species he tested (from 11 families) would consume dead prey offered in a lab situation. Thus, Sandidge's (2003) conclusion that brown recluse spiders are unique in their ability to "actively scavenge," or that their behavior is typical in a natural setting, has been questioned.

Despite the sporadic work on brown recluse behavior over the last fifty years, there are still no published detailed behavioral studies of brown recluse spiders in the field. This study aims to begin to fill this gap and answer some basic questions about recluse spider behavior *in situ*. Specifically, I observed a synanthropic population of brown recluses to quantify daily activity patterns and seasonal activity, including prey choice. Additionally, I conducted *in situ* tests on scavenging behavior.

METHODS

Activity patterns and feeding.—Under red light, I observed brown recluse spiders in a large (20 × 10 m) urban garage in Monmouth, IL, USA (40.19° N, 90.64° W) from June 2013 to June 2014 when they were active (May–October). The unheated garage, 100+ years old, has double brick walls 3.3 m high with loose mortar between the two layers but no insulation and a gently sloped, wood roof peaking at 4.8 m covered by standard asphalt shingles. Although the garage has several openings for windows all but one are covered either by boards, sheet metal or both. The single glass window has a partially drawn blind and admits minimal light from the

north. The west garage door is used by the owners for their two cars and is typically left open during the day. Three vintage cars and a boat are stored in the east side of the garage and moved only once a year (in mid-summer) through a separate garage door on the east wall. In addition to the usual peripheral clutter typical of a household garage, next to the north wall there are two small (approximately 2 × 1 m) piles of wood and debris. Spider activity was concentrated on the peripheral floors and walls except by the garage door that was opened daily.

I focused on nocturnal observations which I divided into three observation periods of 3 h each from 21:00 to 06:00 h and supplemented with similar daytime observations. During observation periods, I alternated between focal animal sampling and scan sampling. In 10–15 minute periods, I watched groups of focal animals that ranged from 5–10 individuals (high densities and low activity allowed for more than one focal individual to be observed simultaneously). I recorded all activity to the nearest 15 sec and identified any prey items. In 45 hours of focal sampling on 15 separate dates between June and August, 2013, I totaled 278 spider-hours (one hour observing one spider) of observation. For statistical analysis and graphing, I combined focal data into eight, three-hour time periods (using start time of the focal sampling period).

Additionally, every ten minutes (between focal sampling periods), I patrolled a designated path of approximately 20 m in the area of the garage with the most spiders and scan sampled additional spiders, categorizing behavior as sedentary, web-maintenance, walking, feeding, or mating. Because spiders were not marked, some individuals were repeatedly observed. Collectively, however, I observed up to 50 individual spiders in a given 3-hour sampling period by both methods. The mean number of spiders observed by both methods was 24.4 (SE = 3.2, range 12–56), or 1.2 spiders/m^2, although densities in certain areas could reach 5–6 spiders/m^2. I conducted additional scan sampling outside of these 3-hour sampling periods in the fall and early spring for a total of 5700 scan observations of individual spiders on 28 separate dates. After analysis, I found scan observations to be a less labor-intensive yet still accurate portrayal of activity in this species. Because more observations on more individuals could be made, observations on feeding, mating, and other relatively rare activities were more likely to be made with scan sampling. I used chi-square tests to compare proportions of time spent in the various activities across sex and age. I conducted one-way ANOVAs (after arcsine transformation) to compare percent time spent in various activities across time of day and date (season).

I divided predation events into feedings observed in progress and attacks, further subdividing attacks into captures, failures, or rejections. If the prey was approached and touched, but the spider did not make an attempt at capture, I classified it as a rejection; if the spider continued to pursue but the prey evaded capture, I classified it as a failure.

Scavenging tests.—I used 3-week old crickets (*Acheta domesticus*) supplied by Fluker Farms (Port Allen, LA) that I killed by freezing. After thawing to room temperature, I placed single crickets either approximately 0.5 m or 1 m from resting recluses 90 min after sunset. I returned after 1 h and again after 12 h to record if a spider was feeding on the prey. In 95% of cases, the cricket was either untouched or being fed on by a recluse spider. In the few instances (n = 8) when prey had disappeared after 12 h but a spider was not observed, for purposes of this test I assumed a brown recluse spider had discovered the prey and taken it out of sight to a retreat. I observed several instances where recluse spiders had moved the prey up to 30 cm. Although some disappearances may have been due to ants, which I observed disassembling and removing prey in one instance, I saw no evidence of mice or other scavengers or predators in the garage interfering with these tests. I used chi-square tests of independence to examine the effects of distance (of prey from a spider) and time of exposure on scavenging frequencies.

RESULTS

Activity patterns.—Overall, scan observations reveal that recluse spiders spent 88% of observed time sitting in wait for prey. Considering that reeluses do not move during feeding, recluses spent 92% of their time motionless. Web maintenance (8%) consisted of slow, methodical movements laying down new strands of silk in a roughly circular area typically less than 30 cm in diameter. Other activities such as walking distances greater than 0.5 m, mating, and agonistic encounters were rarely observed. Focal observations reveal nearly identical statistics with 87% of observed minutes sitting in wait for prey, and 92% either waiting or feeding.

Influence of sex and age on activity: Male and female brown recluses showed the same level of overall activity (χ^2 = 2.1, df = 1, P = 0.15), with females slightly more active (17.3%) than males (13.7%). However, much of the slight difference in female activity was due to their higher incidence of feeding. Removing feeding effeetively eliminated any minor difference between male (11.3%) and female activity (10.6%). In contrast, juveniles were much less active than adults, spending scarcely 5% of their time active (χ^2 = 54.0, df = 1, $P < 0.0001$).

Diel patterns: Scan observations showed that recluses were largely, but not exclusively, nocturnal. Fewer recluses were observed during the day, and they were even more sedentary (Fig. 1A). Nocturnal activity levels averaged nearly 14% while daytime activity averaged approximately half that (χ^2 = 35.6, df = 7, $P < 0.0001$). Although recluses may feed for hours on a single prey item, removing feeding as an activity did not change these diel patterns (χ^2 = 32.4, df = 7, $P < 0.0001$). Most of the individuals observed to be active during the day were found in the darker recesses of the garage, far from the door which was typically left open during daylight hours.

Focal observations confirmed the basic pattern of higher activity at night (Fig. 1B), particularly from 0:00–6:00 h. Recluses were nocturnally active for about 13% of observed minutes compared to less than 8% activity during daylight hours (ANOVA, F = 6.8, df = 7, $P < 0.0001$) and this trend was more pronounced when removing feeding activity (F = 8.0, df = 7, $P < 0.0001$). Much of the observed daylight activity was feeding, probably on prey captured at night with protracted feeding times extending into daylight hours.

Seasonal patterns: Spiders were not active at all between mid-October and mid-April, but presumably were hibernating in abundant refugia within the garage (e.g., in large, deep cracks in the foundation, under or in stored items). From scan

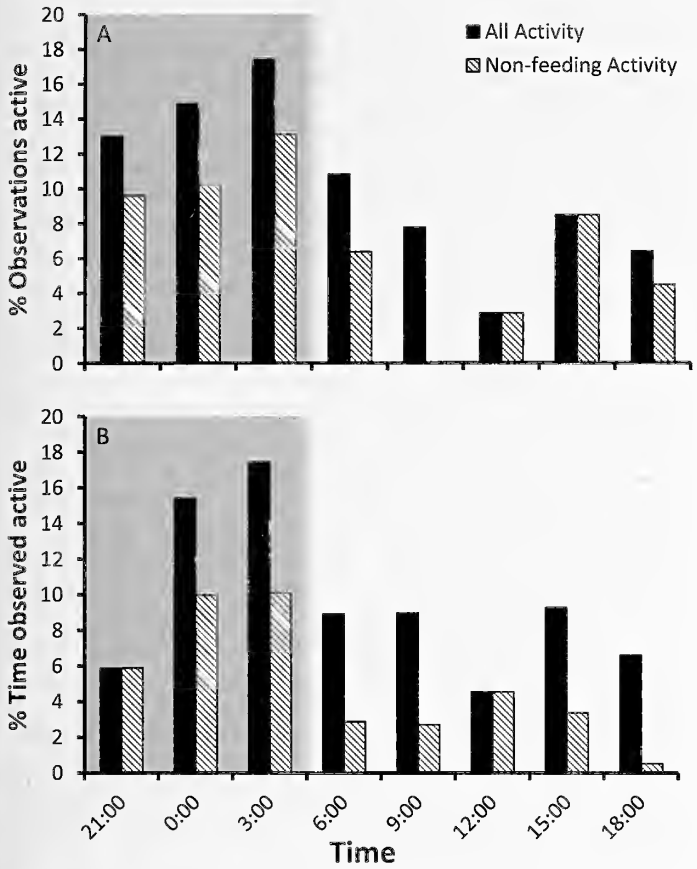

Figure 1.—Diel activity patterns in brown recluses. A. Scan observations. B. Focal observations. Shaded area corresponds to approximate nocturnal hours.

observations over the 5 months of peak activity divided into 2–week periods, brown recluses showed significant seasonal variation in activity (χ^2 = 32.3, df = 9, P = 0.0002). While their activity seemed somewhat periodic (Fig. 2), activity varied from an average of 11–17% and was not correlated with daily high (r = – 0.16, P = 0.42) or low (r = – 0.07, P = 0.73) temperatures (Illinois State Climatological Office reeords for Monmouth, IL). Similarly, lunar cycles did not correspond to peaks or troughs in activity.

Mating and agonistic interactions.—I observed 19 mating attempts (four during focal sampling and 15 in scan samples) and of these three failed. In the four focal mating attempts (where the entire interaetion was observed) the total interaction time varied from 2:20 to 9:00 min. Precopulatory behavior consisted of tapping with the forelegs, stroking of the female with forelegs, and drumming or stridulating with palps as previously described (Hite et al. 1966; Horner & Stewart 1967). One to four intromissions were typical in a single mating attempt, although in one pair 13 intromissions occurred. Notably, the male in this interaction was in very poor condition with a visibly shriveled abdomen. The duration of intromissions varied from 1 to 13 sec with a standard deviation (3.1 sec) greater than the mean (3.0 sec). Briefer intromissions of 1 sec or less appeared to be tentative approaches before actual sperm transfer during the final, and usually longest, intromission. In only one case was the final intromission shorter than prior attempts. The final

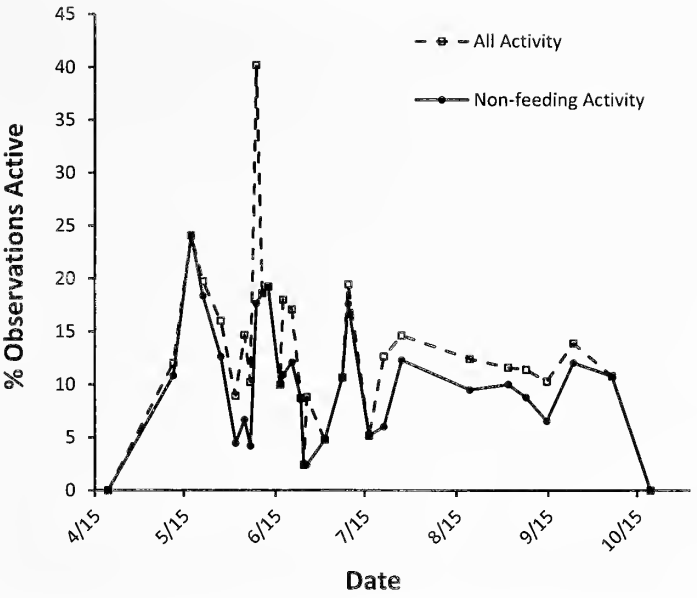

Figure 2.—Seasonal activity patterns in brown recluses from scan observations.

intromission averaged 5.8 sec, significantly longer than preliminary attempts averaging 1.7 sec (t = 3.98, df = 15, P = 0.001). After the final intromission, the male quickly separated and ran rapidly to 0.5 m or more away from the female.

Only five agonistic encounters were observed, four of these during focal animal sampling. Encounters were typically very brief, lasting less than five seconds and involving tapping with the forelegs, often followed by a rapid retreat of one individual, in two cases, the much smaller spider.

Feeding.—I observed 81 prey encounters (spiders feeding or attacks in progress), 63 during scan sampling and 18 during foeal sampling. While I observed fewer predation events in focal sampling, more actual attacks were recorded by this method. Of all prey encounters observed by both methods, most observations were of spiders already feeding (n = 64) while the remainder (n = 17) were in the midst of attacks or approaches.

The most common prey items successfully captured or being fed upon were other spiders (25%, n = 17) with other brown recluses (n = 7) the most prevalent. Beetles (principally Carabidae) were nearly as frequently preyed upon (21%). Wood lice (Isopoda) were also commonly encountered but were less successfully preyed upon (15%), while crickets (Orthoptera) and moths (Lepidoptera) were also often victimized (13 and 9% respectively). Of the 17 attacks observed in progress, only three prey were successfully captured while three were rejected. In the 278 hours of focal observations, I never observed a spider scavenging on naturally available dead prey.

Scavenging trials.—Of all prey offered (n = 146), spiders found only one in six (17%). One in four prey (24%) plaeed within 0.5 m of a visible spider were found within 12 hours, but less than half that (11%) were discovered if placed 1 m from a visible spider (χ^2 = 4.2, df = 1, P = 0.04). Many more prey were found after 12 h (14%) than after 1 h (3%) at both

distances (χ^2 = 16.9, df = 1, P = 0.0002). On several occasions, a cricket placed within 0.5 m of multiple spiders (up to four) was left untouched. I observed no differences in scavenging frequency between males (n = 6) and females (n = 8) or juveniles (n =11) and adults (n = 14).

DISCUSSION

The brown recluse spider's overall low levels of activity are consistent with its sit-and-wait predation lifestyle and prior studies on its longevity (Hite et al. 1966; Elzinga 1977), hardiness (Eskafi et al. 1977) and extremely low metabolic rate (Carrel & Heathcote 1976). In light of this study, the common knowledge that the brown recluse spider is largely nocturnal is perhaps better described as negatively phototaxic, as spiders in this study were often observed to be active during the day in low light conditions. Given the low cost of their inactive lifestyle, being out of a retreat during the day but still in darkness (e.g., caves, garages, attics, basements) would probably cause little increase in predation risk, particularly because nearly all the mortality that I observed was due to intraspecific predation.

The similarity in male and female activity levels contradicts conventional wisdom that males wander more and widely as they are often caught in sticky traps. However, my observations are biased against capturing many long-distance movements, which could be infrequent, but significant. Also, the few long movements I did observe were, in fact, mostly males. Studies of individually marked spiders currently underway should shed more light on infrequent, but longer distance movements by recluses. Juvenile activity may be far lower simply as a result of their smaller size and thus smaller webs to construct and maintain. Alternatively, juveniles may also be more at risk of death due to starvation so that movements are avoided as a means of conserving energy.

The seasonal activity of recluse spiders recorded here (May–October) is consistent with that gleaned from records of submissions of samples from the public which peak between May and August (Vetter 2011b). Vetter (2011b) suggested that the apparently regular seasonal activity of recluses even in temperature-controlled human habitat indicated a photoperiod dependency. Lending some support to this hypothesis, in this study brown recluses were not abundant until at least four weeks after nighttime temperatures had increased to 10°C, well above that of the 5°C minimum required for activity according to Hite et al. (1966).

The mating behavior I observed was similar to that recorded by Hite et al. (1966) in the lab. They recorded multiple intromissions (up to 11 times) as well, with some lasting 20 to 30 sec. Horner & Stewart (1967) also found that pairs would engage in up to nine intromissions, but did not report durations. Curiously, in one of the four mating attempts that Hite et al. (1966) detailed, the duration of intromission decreased with each subsequent attempt, the opposite of what I observed in six of nine interactions with multiple intromissions.

Considering the high density of spiders in some areas, the low number of agonistic encounters reflects the sedentary nature of brown recluses, and possibly mechanisms whereby they can avoid other individuals when they are moving. However, the relatively high rate of intraspecific predation (seven instances in 67 successful predation events) suggests that while agonism is rare, it can have significant consequences. Stropa (2007), in staged lab encounters of *L. gaucho* males, found that only 22% of interactions were aggressive (lunges or bites); the vast majority of interactions (77%) were non-aggressive. He attributed this to the dense populations of the spider and possible sociality. Vetter & Rust (2008) also noted that agonism among juveniles reared in the lab is rare if they are supplied with ample food. Alternatively, in a non-lab situation where food is scarce, generally low levels of aggression could be a mechanism whereby smaller spiders reduce their risk of mortality from intraspecific predation.

Like most spiders, brown recluse spiders appear to be opportunistic predators. In this study, recluse spiders fed on conspecifics and other spiders, isopods, beetles, crickets, and moths. These arthropod groups were also the potential prey that I most commonly observed in the garage. Hite et al. (1966) reported a wide variety of arthropods found in webs of brown recluses without commenting on relative frequency of prey or their abundance. Richman (1973) studying a related species (*L. arizonica*) found principally ants captured in webs, which were also very common at his study site in the Sonoran desert. Fischer et al. (2006) also reported that isopods and beetles were common prey in webs of the South American species *L. intermedia* in both homes and forested habitat. In contrast to my study, arachnids were rarely reported (though commonly collected nearby) as prey, and various hymenoptera, especially ants, were common prey.

Since Sandidge's (2003) assertion that brown recluses "actively search for dead prey" and actually prefer dead to live prey, two publications have cast doubt on his hypothesis. Cramer (2008) replicated Sandidge's (2003) lab tests taking into account three influential variables: live prey size, dead prey quality, and the spider's level of hunger. I found that brown recluse spiders preferred live over dead prey, fresher over more decomposed prey when scavenging, and that starved spiders would take more risks attacking large, live prey than would well-fed spiders. My observations of predation in the garage showed that recluse spiders were not hesitant to attack live prey (though often unsuccessfully), contrary to Sandidge's (2003) findings that recluses often fled from live prey. Combining these findings with Vetter's (2011a) observations that 99% of non-*Loxosceles* spider species he offered dead prey in a lab situation would consume it, and observing that brown recluse spiders seldom move more than half a meter in a night led to my *in situ* tests on scavenging. My tests confirm that while brown recluse spiders will scavenge given the opportunity, the likelihood of them encountering dead prey is low unless the prey dies very close to a resident spider. As suggested by Sandidge (2003), it is conceivable that a large influx of dead prey from pest control efforts could provide a pulse of potential prey to a dense population of brown recluse spiders. However, given that 1) spiders found only 20% of dead prey in this study, 2) the desirability of dead prey declines rapidly with age (Cramer 2008), and 3) a single prey item is often sufficient to take a recluse spider through its next molt such that many dead prey killed by pesticide application would be of low quality after a few days, the potential impact of such a scenario on overall population size

is far from certain without controlled, manipulative field experiments on multiple populations.

While this study focused on a single synanthropic population in an urban garage, my observations support anecdotal reports on the behavior of this species and it seems that some general conclusions can be made with respect to spiders living in association with humans. Brown recluse spiders are sit-and-wait predators that expend very little energy on prey capture beyond the costs of web construction and maintenance. Once they emerge from winter retreats, levels of activity do not vary predictably across seasons. Likewise, there is no pattern in their nocturnal activity, and they can be and are active diurnally under low light conditions. Mating behavior in the wild is similar to that recorded in the lab, and agonistic encounters are rare. Brown recluse spiders appear to be non-selective predators that will consume whatever live arthropod prey stumble into their webs that they are capable of subduing. They do not actively search for either live or dead prey, but will feed on dead prey if they discover it in their immediate vicinity. Future behavioral observations of recluse spiders in natural habitats would be a welcome contribution to knowledge of this medically important species.

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Sexual cannibalism in the spider *Alpaida veniliae* (Keyserling 1865) (Araneae: Araneidae)

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Abstract. Postmating cannibalism where a female attacks, kills and consumes a male after a sexual encounter is frequently influenced by certain male morphological and behavioral characteristics. We conducted behavioral assays in the laboratory to test the predictions that male *Alpaida veniliae* (Keyserling 1865) with larger absolute and relative size in relation to their mate and those having longer courtship and copulation duration would have lower probability of being cannibalized by females after a sexual encounter. We performed a set of mating trials exposing males of different sizes to virgin females. We observed copulation in 88.8% of mating trails; its duration was very brief compared to courtship. Only a few attempts (16.7%) of recopulations with the same female were recorded, and in all these cases the first copulation was significantly shorter than the mean copulation duration of those who had only one copulation. The percentage of postcopulatory cannibalism was 47.6%. There was no correlation between the relative and absolute male size and duration of courtship and copulation. Postcopulatory cannibalism was independent of courtship and mating durations but was affected by absolute and relative male size. Smaller males were more frequently cannibalized than large ones. However, it remains unclear whether sexual cannibalism in *A. veniliae* may be explained by female mate choice or whether smaller males are less able to escape or defend themselves. More studies are needed to understand the underlying factors of postcopulatory cannibalism of *A. veniliae*, as well as to elucidate their possible ecological and evolutionary implications.

Keywords: Sexual behavior, courtship, copulation, postcopulatory cannibalism

Sexual cannibalism is a common behavior in some spider families, including Araneidae, Theridiidae and Pisauridae. In this process, the female attacks, kills and consumes a male before, during or after a sexual encounter (Elgar 1992; Elgar & Schneider 2004; Schneider & Andrade 2011). Many ecological factors such as food availability, population density, sex ratio, and body mass of individuals affect the selective benefits of cannibalism (Wilder et al. 2009). The adaptive significance of sexual cannibalism is often sex-specific because the costs and benefits for males and females can differ significantly, and depends on the time of occurrence during sexual interaction and if the mating system is monogamous or polygamous (Newman & Elgar 1991; Elgar 1992; Andrade 1996, 1998; Elgar & Schneider 2004).

Differential sexual cannibalism takes place when females respond differently to certain conspecific males according to some measurable morphological or behavioural trait. However, to date, studies where cannibalism occurs during or after copulation do not provide strong evidence that differential postcopulatory cannibalism occurs (Prenter et al. 2006). Male size, both absolute and relative to female size, often predicts the likelihood of cannibalism. Wilder & Rypstra (2008) tested for a relationship between sexual size dimorphism and sexual cannibalism within and among species of spiders and found that cannibalism was more likely when males were much smaller than females. Elgar & Nash (1988) observed in *Araneus diadematus* Clerck 1757 that sexual cannibalism was determined by male body size. While females attacked males of different sizes at approximately the same rate, relatively larger males were better than smaller males at resisting attack. Roggenbuck et al. (2011) also reported that smaller males of *A. diadematus* were more likely cannibalized than the larger ones. Similarly, in the wolf spider *Pardosa pseudoannulata* (Bösenberg & Strand 1906), Lingbing et al. (2013) found that there was a strong positive relationship between mate size dimorphism and the occurrence of sexual cannibalism. In contrast, mating advantages for smaller males are reported in *Argiope keyserlingi* Karsch 1878 (Elgar et al. 2000), and in *Nephila edulis* Labillardière 1799 (Schneider et al. 2000).

Courtship behavior may function to reduce cannibalism by the female. During courtship, both sexes can transfer information on species identity, mating status and quality (Wignall & Herberstein 2013). Andrade & Banta (2001) experimentally manipulated courtship duration in the sexually cannibalistic redback spider (*Latrodectus hasselti* Thorell 1870) and did not find any difference in size, mass or condition between cannibalized and noncannibalized males, but noted that males exhibiting short courtship had lower mating success and experienced more female rejection behavior than did males with long courtship.

Another factor that seems to be related to postcopulatory cannibalism is copulation duration which has long been thought to be one of the most important measures of male reproductive success (Simmons 2001). While it is presently recognized that postcopulatory cannibalism can extend or shorten the duration of copulation for cannibalized males compared to survivors, specific details of female discriminatory behavior remain unclear (Prenter et al. 2006). Although postcopulatory cannibalism was not significantly related to any male morphological trait in the orb-weaving spiders *Argiope aurantia* Lucas 1833 and *Argiope bruennichi* (Scopoli 1772) (Fromhage et al. 2003; Foellmer & Fairbairn 2004; Schneider et al. 2006), it was correlated with prolonged copulation duration. Moreover, Andrade (1996) showed that males of *Latrodectus hasselti* were cannibalized during or after copulation and those that were cannibalized had longer

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copulations and fertilized a larger proportion of the eggs than males that were not eaten.

Alpaida veniliae (Keyserling 1865) (Araneae, Araneidae) is one of the most abundant orb weaving spiders in transgenic soybean crops in Buenos Aires province, Argentina. Our previous studies have focused primarily on biological and ecologieal attributes that indicate *A. veniliae*'s importance as a predator of soybean crop pests (Minervino 1996; Benamú et al. 2011). *Alpaida veniliae* is moderately size–dimorphic and most females are monogamous. Laboratory observations (Benamú et al. 2012) described the process of courtship, mating and postmating behavior of this species. Courtship and copulation occurred in the capture web of the females, and courtship represented 75% of the total duration of a sexual encounter with a male. These observations suggested that male courtship is critical for recognition by the female and for avoiding female aggression. Precopulatory cannibalism was not observed in *A. veniliae* and 50% of males were cannibalized post-copulation. There were no genital plugs and during copulation the male inserted only one of the two pedipalps into only one of the paired copulatory ducts of the female. The males did not try to insert the second palp.

Although several studies have investigated many potential causes of cannibalism separately, there is a paucity of studies that tested multiple causes simultaneously. Here we conducted behavioral assays in the laboratory to test the predictions that males with larger absolute and relative size (male/female ratio) and those having longer courtship and copulation durations would have lower probability of being cannibalized by females after a sexual encounter.

METHODS

Study system.—We collected adult gravid females and adult males of *A. veniliae* in transgenic soybean crops located in Chivilcoy (35°01′ S, 60°06′ W), Buenos Aires, Argentina, and reared them at 25 ± 2 °C, 75 ± 5% RH and a photoperiod of 16:8 (L:D). Egg-sacs were incubated and spiderlings were reared until adulthood. Juveniles and adults were transferred to 500 ml transparent glass jars. Once a week, juveniles were fed *ad libitum* with *Drosophila melanogaster* and subadults and adults were fed with *Musca domestica*.

Experimental protocol.—Adult virgin females collected at random from the colony were placed individually in glass frames (15 × 10 × 5 cm) to allow web building. The female was placed in the cage three hours before introducing the male, and fed with one *Musca domestica* immediately before the experiment to avoid cannibalism elicited by hunger. We selected virgin and non-virgin males from the colony, choosing individuals that differed conspicuously in size (total body length). Males were assigned at random to each female and each was set on the bottom of a frame so it could detect the female's pheromones on the threads of the web (n = 116 pairs). At the end of the experiment individuals were preserved in 75% ethanol. We measured body length of each individual using an optical stereo microscope (NIKON SMZ-10) with an ocular micrometer and calculated the relative size (male/female ratio).

During mating trials, we defined a male as being cannibalized if the female wrapped it in silk after copulation. In these cases, the mating pair was immediately removed and killed so that we could measure the male before the female could consume it. If, after copulation, the female and male unhooked and the male jumped away from the female, we interpreted this as being no cannibalism. The mating pair was removed, killed, and measured.

Durations of courtship, first and second copulations, and palp insertion and the occurrence of post-mating cannibalism were video-recorded using a SONY HDR-XR160 High-Definition Handycam with 30X optical zoom. Recorded data were analyzed by Pinnacle Studio 9 (version 3.8).

Voucher specimens.—All specimens of *A. veniliae* used in this study were deposited in the insect collection at the Museum of Natural Sciences of La Plata (UNLP), Argentina.

Statistical analysis.—To assess for differences in body size between male and female, as well as in duration of courtship and copulation between the first and second copulation, paired Student t tests were used. The frequency of insertion of the right or left palp during copulation was analyzed by chi-square contingency test. To examine the dependence between the absolute and relative male size (male/female ratio) and duration of courtship and copulation, the Pearson correlation coefficient (r) was used. To test the effect of absolute and relative male size (male/female ratio) and duration of courtship and copulation on postmating cannibalism, we used generalized linear models (GLM). A multiple regression model that explicitly assumes binomially distributed errors and a logit link function was used (McCullagh & Nelder 1989). Before fitting the model, explanatory variables were checked for multicollinearity. Cannibalism was considered a categorical (binary) response variable, and courtship duration, copulation duration, male body size, and male/female size ratio as explanatory variables. The model was fitted with maximum likelihood, and the statistical significance of each variable was tested in turn by a stepwise procedure, and the Wald statistic was used to test the significance of each regression coefficient. Statistical analyses were performed using Statistica v. 7 (StatSoft 2007), and in all tests, $P < 0.05$ was considered significant.

RESULTS

Alpaida veniliae females were significantly larger than males (t = 22.46; df = 204; $P < 0.001$); body length (mean ± SE) was 6.08 ± 0.03 mm (n = 103) for females and 5.07 ± 0.03 (n = 103) mm for males. The ratio male/female body length (mean ± SE) was 0.84 ± 0.009 (n = 103).

Spider pairs copulated in 103 (88.8%) out of 116 observed trials. Males started courtship with alternating constant and intense vibratory movements of the third pair of legs on the threads of the female's web. If the female did not react aggressively, the male construeted a mating thread at the edge of the orb web. Then, males engaged in a series of vibratory movements with their legs and abdominal shudders on the mating thread with no direct female contact. The female then advanced over the mating thread, slipped down, apparently being held only by the third and fourth pairs of legs, and adopted the receptive posture. Male and female touched each other repeatedly with their first and second pair of legs, while the male performed abdominal shaking and rubbed the female epigyne with his palps until finally assuming the copulation position. Duration of copulation was very brief compared to

Table 1.—Correlations between absolute and relative male size and duration of courtship and copulation in *Alpaida veniliae*.

Variable	*r*	*n*	*P*
Male size			
courtship	0.079	105	0.419
copulation	−0.109	105	0.265
Male/female ratio			
courtship	0.033	103	0.734
copulation	0.085	103	0.390

courtship; mean duration was 10.99 ± 0.11 (n = 103) seconds for copulation and 278.05 ± 6.54 (n = 103) seconds for courtship, and they were not correlated with either the absolute male size or the relative male size (male/female ratio) (Table 1). Males inserted a single palp during copulation and the right one was used more often than the left one (χ^2 = 25.81; $P < 0.001$).

When the male was not cannibalized after mating, both sexes unhooked from each other quickly and the male jumped out of the female's reach, cut the mating thread and left. The female stopped being receptive and kept the male away with aggressive attacks or by eating its own web, preventing a new courtship by that male or others.

Premating cannibalism was never observed in 103 mating trials. The percentage of postcopulatory sexual cannibalism, i.e., trials in which females devoured males after copulation, was 47.6% (n = 49). We recorded nine attempts of a second copulation. In all nine pairs, the first copulation lasted 6.22 ± 0.27 seconds, which was significantly shorter than the mean duration of copulation of those who had only one copulation (t = 11.35; df = 110; $P < 0.001$). When the male attempted to copulate again with the same female, it used the other palp and repeated the courtship, which was significantly longer (603.22 ± 22.5 seconds) than the first (t = 3.92; df = 112; $P < 0.001$). Duration of the second copulation was very similar (11.67 ± 0.3) to the duration of the first copulation of males that did not remate (t = 1.604; df = 112; P = 0.111). Percentage of postcopulatory sexual cannibalism after remating was 55.6% (n = 5).

Postcopulatory cannibalism was not related to the duration of either courtship or copulation (Table 2). By contrast, absolute and relative male size significantly affected postcopulatory cannibalism. Postcopulatory cannibalism was inversely related to male size and to male/female size ratio. Thus, the larger the absolute size of the male and the higher the ratio of male/female size of the interacting pair, the lower the likelihood that the male would be cannibalized.

DISCUSSION

We found that mating behavior of *Alpaida veniliae* strongly resembles those of related araneids (Robinson 1982). We conclude that absolute and relative size of a male in relation to the female affected the occurrence of postcopulatory cannibalism. Smaller males were more frequently cannibalized than large ones. Furthermore, postcopulatory cannibalism was not related to the length of courtship or mating. Sexual cannibalism was common in this species, with ≈ 50% of males cannibalized after their first insemination.

Table 2.—Logistic regression analysis of the probability of cannibalism of the male by the female of *Alpaida veniliae* in the laboratory.

Predictor	Coefficient	S.E.	Wald	*P*
Intercept	12.955	5.435	5.682	0.017
Courtship duration	−0.002	0.003	0.490	0.483
Copulation duration	0.200	0.172	1.344	0.246
Male size	−1.840	0.709	6.739	0.009
Male/female ratio	−6.489	2.629	6.089	0.013

In accordance with our results, there is some evidence that spider females in other species react differently to some conspecific males and that discrimination relies on measurable morphological or behavioral traits such as size and aggressiveness (Prenter et al. 2006; Kralj-Fišer et al. 2012). Prenter et al. (2006), analyzing original data from Rubenstein (1987) in *Metellina segmentata* (Clerck 1757), confirmed that smaller males were cannibalized at a significantly greater frequency than larger males.

The frequency of postcopulatory cannibalism in *A. veniliae* is in agreement with that of other sexually size monomorphic species like *Araneus diadematus* (Roggenbuck et al. 2011). We showed here that post-insemination sexual cannibalism is driven by size differences between the mating partners, a finding that is in accord with the sexual size dimorphism (SSD) hypothesis proposed by Wilder & Rypstra (2008).

As in other orb-web spiders (Maklakov et al. 2003), courtship of *A. veniliae* involved males generating vibrations on the web. Some studies suggest that courtship and signalling during courtship functions to alter the likelihood of cannibalism (pre- or postcopulatory) (Eberhard & Huber 1998; Maklakov et al. 2003; Wignall & Herberstein 2013). Stoltz et al. (2008, 2009) reported that, when males of the redback spider *Latrodectus hasselti* compete with rivals in the presence of females, those that exhibited shorter courtship were cannibalized before mating was completed, while longer courting males were able to inseminate both sperm storage organs. Females employ premature cannibalism to reduce the paternity of males that had reduced investment in courtship if they were clearly distinct from their rivals. Although in our experiments courtship was the most prolonged part of the sexual activity, its duration was not related to postcopulatory cannibalism. Given that our trials were carried out using individual pairs, we are unable to speculate about whether the presence of other males within a competitive context might affect the cannibalistic response of *A. veniliae* female to the duration of male courtship.

The brief copulation observed in *A. veniliae*, possibly due to the insertion of a single male palp, is similar to that of *Argiope bruennichi* (Schneider et al. 2005, 2006). Given the duration of copulation and that females mate only once raises the question of whether this time is enough for a complete fertilization. According to these authors, males of *A. bruennichi* can transfer 50% of their sperm in the 10 seconds, which is the duration of the whole mating, and even during an average mating of 8 seconds they can transfer enough sperm to ensure a full fertilization. As the duration of mating in *A. veniliae* is similar to that of *A. bruennichi*, it is also possible that a complete fertilization takes place during mating. Additionally, when Snow & Andrade (2004) tested duration-dependent sperm

transfer in the redback spider, *L. hasselti*, the found that although copulations ranged between 5 and 20 min, the redback males transferred the majority of their sperm within the first 5 min of copulation.

Interestingly, second copulations took place only when the duration of the first mating was less than 10 seconds, suggesting that this length of time was too short for a complete insemination. In these cases, and probably to overcome the resistance of the female, the male had to perform a significantly longer courtship for a successful second copulation.

We demonstrated that absolute and relative male's size affected postmating cannibalism, although from our data we cannot conclude whether sexual cannibalism in *A. veniliae* is a mechanism of mate choice or whether smaller males are less competent to escape or defend themselves (Elgar & Nash 1988; Elgar 1992; Persons & Uetz 2005; Prenter et al. 2006). Sexual conflict in this species should be lower compared with those that have premating cannibalism. However, further studies are needed to understand the underlying factors of postcopulatory cannibalism in *A. veniliae*, as well as to elucidate their possible ecological and evolutionary implications.

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Dramatic histological changes preceding suicidal maternal care in the subsocial spider *Stegodyphus lineatus* (Araneae: Eresidae)

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Abstract. Parental care entails physiological costs to the mother. These costs, even if dramatic, are usually reversible and do not result in mortality of the mother. In the spider *Stegodyphus lineatus* Latreille 1817 (Eresidae), maternal care is extreme and irreversible: mothers regurgitate food for the young and then die when consumed by them (matriphagy). We examined whether the mother's midgut tissues undergo structural changes in preparation for regurgitation and matriphagy. Our histological data show that the midgut diverticula (MD) tissues start to degrade during the egg sac incubation period. When the young emerge from the egg sac, the midgut tissues are partly liquefied and are retained within the MD. The degradation process intensifies when the female feeds her young by regurgitation and liquid tissue is observed within and among the diverticula lobes. The presence of the lumen of a diverticulum during the regurgitation process suggests that degenerated tissues enter the lumen and form the regurgitated fluid. At matriphagy, the abdomen is filled with liquid containing nutritional vacuoles, which the young imbibe after piercing the female's abdomen. We conclude that the MD undergoes a gradual degradation process that maximizes the nutritional potential of the female's body and finally enables complete consumption of her soma. These changes are consistent with the extreme semelparous reproductive system of *S. lineatus*, where a female invests all of her resources into a single reproductive event. This is the first demonstration of the mechanism underlying suicidal maternal care in an arthropod.

Keywords: Matriphagy, midgut diverticula, nutritional vacuoles, regurgitation, tissue degradation

Raising offspring is costly, and investment in parental care may be traded off against future reproduction (Fox & Csezak 2000). A major cost of parental care is the physiological burden on the mother. Maternal care in spiders may be transient or extended (Yip & Rayor 2013). Transient maternal care is widespread in spiders and is mainly restricted to protection of egg sacs or of the young until dispersal shortly after emergenee (Lubin & Bilde 2007). Extended maternal care of offspring after emergence from the egg sac (subsocial behavior) has evolved multiple times, and occurs in at least 65 species in 16 families (Yip & Rayor 2013). The behaviors include feeding the young with captured prey (Gundermann et al. 1988; Schneider 1996), producing trophic oocytes (Gundermann et al. 1991; Evans et al. 1995), regurgitating food for the young (Kullmann & Zimmerman 1975) and matriphagy, i.e. the consumption of the mother by her young (Seibt & Wickler 1987; Kim & Horel 1998; Kim et al. 2000). While transient maternal care does not restrict the production of additional broods, extended maternal care, such as providing prey for the young, regurgitation feeding, and production of trophic eggs involve prolonged association between the mother and her brood and may constrain the female to a single clutch (Yip & Rayor 2014). Matriphagy is an extreme form of maternal investment and is an irreversible dead-end for the mother that precludes the possibility of future reproduction. Do such constraints on future reproduction involve changes in physiology and internal anatomy that at some stage become irreversible?

Female *Stegodyphus lineatus* Latreille 1817 (Eresidae) exhibits intensive maternal care for newly emerged young, first by regurgitating liquid food and later by allowing matriphagy. Females lay a single clutch of ~80 eggs that constitutes less than 3% of the body mass of the mother at oviposition (Schneider 1996), and will lay a second clutch of eggs only if the first is lost due to predation or male infanticide (Schneider & Lubin 1997a). After emergence of the young, the female provides the young with regurgitated fluid (Kullmann & Zimmermann 1975). Young of females fed with radioactive flies became radioactive a few days after emergence, indicating a transfer of digested prey from the mother to her young (Kullmann 1972). After two weeks of regurgitation feeding, the young climb on the mother's body and consume her (matriphagy; Seibt & Wickler 1987); within 2–3 hours they extract her body fluids leaving behind only a dry exoskeleton (Salomon et al. 2005). In this species, the mother ceases to eat after the young emerge. She does not provide prey to the young and the young are incapable of catching and ingesting prey independently, and thus they will die without her regurgitated fluid (Salomon & Lubin unpubl. data). Therefore, the body mass of the female at the emergence of the young constitutes all the food available for the young during maternal care. During regurgitation, the female loses 41% of her body mass, while in matriphagy, the young consume an additional 54% of her body mass measured at emergence of the young. Thus a total of 95% of her body mass is provided to the young as food (Salomon et al. 2005). These maternal care behaviors lead to a threefold increase in the body mass of

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the young from emergence until dispersal (Salomon et al. 2005). After matriphagy, the young remain in the maternal nest for approximately two weeks, undergo another molt, and only then start to disperse gradually (Aviram 2000).

Provisioning of food by regurgitation and matriphagy are linked to the reproductive stage of the female. Schneider (2002) showed that unmated *S. lineatus* females and females after egg-laying will not regurgitate food to foster young, but once females have young of their own, they will feed foster young of other females. One possible interpretation of this finding is that females are physiologically incapable of regurgitating to the young if they are not in the appropriate reproductive stage, namely with young of their own. Matriphagy, like regurgitation, occurs only when the female is in the right reproductive stage. In the spider *Amaurobius ferox* Walckenaer 1820 (Amaurobiidae), matriphagy is initiated only after reciprocal communication between mother and young through web vibrations (Kim & Horel 1998). Females after egg-laying and females with young, but not virgin females, were all consumed when experimentally introduced to foster young ready for matriphagy. However, only females before matriphagy exhibited the web vibration behaviors that activated the young and directed them towards her abdomen (Kim & Horel 1998). The evidence that both regurgitation feeding and matriphagy are closely linked to the female's reproductive stage suggests that internal changes are necessary well in advance to prepare the female for these functions. In the present study we asked: What are these changes and when do they occur in the reproductive sequence from maturation to matriphagy?

We used histological sections to examine the changes occurring in the opisthosoma (abdomen) of *S. lineatus* females at different reproductive stages. The digestive system of araehnids is divided into foregut (pharynx and esophagus), midgut, and hindgut (Bertkau 1885, cited in Ludwig & Alberti 1988; Collatz 1987; Klann & Alberti 2010). The midgut of spiders consists of the midgut tube and numerous blind branches called 'midgut diverticula' (henceforth, MD), located mostly in the opisthosoma (Ludwig & Alberti 1988, 1990; Laino et al. 2009). These diverticula function as a digestive and storage organ (Collatz 1987). The MD consists of a dimorphic epithelium containing secretory and digestive cells (also called 'resorption cells') connected by intermediate tissue (Bertkau 1881, 1885, cited in Ludwig & Alberti 1988; Nawabi 1974; Ludwig & Alberti 1990). Spiders use extra-oral digestion to obtain nutrients from prey, regurgitating an enzymatic digestive fluid into the body of the prey, and then sucking out the liquefied tissues (Collatz 1987; Cohen 1995). The secretory cells containing the digestive fluid empty their content into the lumen of the MD during feeding (Nawabi 1974 for *Stegodyphus pacificus* (Pocock 1900)). One hour after feeding, the digestive cells are filled with nutritional vacuoles containing freshly ingested nutrients (Nawabi 1974; Ludwig & Alberti 1988 for *Coelotes terrestris* (Wider 1834) (Agelenidae)). Segregation of nutrients occurs within these vacuoles, forming droplets of lipids and glycogen that are then extruded from the nutritional vacuoles. Most of the lipid and glycogen droplets are transported to the intermediate tissue for storage (Nawabi 1974; Ludwig & Alberti 1988). Thus the MD is responsible for absorption, synthesis and storage of lipids, and the transfer of energy (Laino et al. 2009). Nawabi (1974) studied the histology of the digestive system of *S. pacificus* females. In her unpublished M.Sc. thesis, Nawabi detailed changes in specific MD tissues (secretion and resorption eells, intermediate tissue and lumen) over periods of time post-feeding. She noted that at the stage of regurgitation-feeding of the young, the midgut tissue underwent a proeess of degradation, and suggested that these tissues were irreversibly transformed into liquid regurgitate for the young. In the present study, we recorded the sequence of ehanges occurring during different stages prior to and during maternal care. In particular, we focused on the process of breakdown of opisthosomal MD tissue during early stages of maternal care, in order to gain insight into the timing of changes in relation to regurgitation feeding and matriphagy.

METHODS

Experimental set-up.—Juveniles, adult females and females with egg sacs of *S. lineatus* were collected from field populations near Lehavim (31° 22′ 04.08″N, 34° 49′ 41.9″E) and in Shagririm forest (31° 19′ 28.45″N, 34° 46′ 44.36″E), Israel. Spiders were maintained at 25–26° C, 14L:10D cycle, and were fed lab-reared adult crickets (*Acheta domestica* Linnaeus 1758) once a week. To examine the MD of females before and during maternal care, we classified females into six reproductive stages (Salomon et al. 2005): 1) sexually mature virgin, 2) post-mating (1 week after mating), 3) mid-egg incubation period (15 days after egg laying), 4) emergence day of the young , 5) mid-regurgitation period (6 days after emergence of the young), and 6) pre-matriphagy (12 days after emergence of the young). Juveniles were raised separately to adulthood in the lab in order to obtain virgin females.

Histological sections.—Three to four females of each stage were sacrificed in order to prepare histological sections. Females of stages 1–4 were sacrificed 3 ± 2 days after feeding. Females in stages 5 and 6 were not fed as they do not capture prey at these stages. Sections from different females of a given stage were checked for consistency. We anesthetized the females with CO_2, made longitudinal incisions in the cuticle of the opisthosoma, and fixed the spiders in 10% formalin. They were then dehydrated gradually in a series of increasing ethanol concentrations and the opisthosoma was cleared and embedded in paraplast (Paraplast Plus: Kendall, Tyco Healtheare, Mansfeld, MA). Cross sections (thickness, 5 μm) were cut onto silane-coated slides (Superfrost plus; Menzel-Gläser, Braunschweig, Germany). With the use of a razor blade, we dissected the opisthosoma of each female into three parts; anterior, median and posterior regions. Five-micron sections were obtained from the anterior and posterior parts while the median parts were sectioned at least 750 microns before collection of sections for staining. All the sections were prepared from anterior, median, and posterior regions of the opisthosoma of each female and stained with Hematoxylin-Eosin. Hematoxylin has a blue-purple color and stains acidic structures such as nucleic acids, while eosin is pink and stains proteins nonspecifically, thus staining a variety of structures in the cell (Fischer et al. 2008). Structures in the histological sections were distinguished based on the literature (Nawabi 1974; Ludwig & Alberti 1990, 1992; Ludwig et al. 1994). Lipid droplets remain unstained due to their extraction by the

ethanol dehydration. Variation in the details of the cross sections among the 3–4 females examined within each stage was minimal, thus allowing us to generalize from the observations. The sections were examined using a Nikon eclipse E600 light microscope and photographed with a Nikon digital camera DXM1200.

RESULTS

The histologieal sections showed that changes occurred in the midgut diverticula (MD) tissue as female reproduction progressed from mating to caring for the young. Figures 1–3 show respectively, the MD tissues as seen under different magnifications, the polarity of changes in the MD with respect to the longitudinal axis of the opisthosoma, and changes in the lumen and other tissues.

Prior to maternal care.—In sexually mature females, the MD were intact and filled up the entire opisthosoma (Fig. 1A). The midgut lumen was surrounded by well-defined, organized lobes (Ludwig et al. 1994) filled with nutritional vacuoles (after Nawabi 1974) and lipid droplets (distinguished by their round, empty form) occur within and among the diverticula lobes (Fig. 1B, C). After mating, the MD remained clearly visible and contained many nutritional vacuoles surrounding the distinct diverticula lobes (Fig 1D–F). Within each lobe, some islets of cells stained dark blue while others stained pink (Fig 1E, F). Tissue degeneration began in the middle of the egg incubation period. Although there was still a clear border between the diverticula and the intermediate tissue, the cell boundaries within both tissues became blurred as the cells and vacuoles started to dissolve (Fig. 1G–I). There was a visible reduction in the number of lipid droplets at this stage.

During maternal care.—At emergence of the young, the borders of the MD became blurred as it continued to degenerate (Fig. 1J–L). The degenerating diverticula and intermediate tissue with their cells and vaeuoles were all condensed into an undifferentiated, liquid tissue that was accumulating among the lobes. The pattern of tissue degeneration was unevenly distributed within the opisthosoma. The tissues began to dissolve primarily at the perimeter of the opisthosoma (Fig. 1J, 2B), near the cuticle, but not interiorly (Fig. 1K). Lipid droplets were apparent among the dissolved MD lobes (Fig. 1L). Vital organs within the body such as the heart and the intestine were unchanged at this stage.

In the middle of the regurgitation period, more of the MD tissue degenerated and fluid containing nutritional vaeuoles was apparent (Fig. 1M–O). Tissue degeneration at this stage resulted in a distinct separation between liquefied tissue and the remaining cellular tissue of each diverticulum within the opisthosoma (Fig. 1N). Contrary to the previous stage where the dissolved tissue was apparent only at the perimeter of the opisthosoma (Fig. 2B), during the regurgitation process, the interior lobes of the opisthosoma were also dissolved, creating patches of liquefied tissue among the dissolving lobes (Fig. 1M). Interestingly, the diverticula lobes surrounding the heart remained intact at this stage (Fig. 1M). Similarly, the muscles, ovaries, and the midgut tube remained intact (Table 1; not shown). The extra-cellular nutritional vacuoles within each degenerating lobe appeared much larger (Fig. 1O) than those at the previous stage (Fig. 1K). The MD lobes that had not dissolved had few lipid droplets and nutritional vacuoles (Fig. 1O).

A few days before matriphagy, the remaining MD tissues appeared granular and condensed, surrounding the still functional heart while the perimeter of the opisthosoma was filled with liquid that contained large nutritional vacuoles (Fig. 1P–R). Few lipid droplets were still observed within the remaining MD tissue.

In addition to the differential degeneration of the MD seen in the median cross-sections of the opisthosoma, there was also an anterior-posterior polarity in the degradation pattern. At emergence of the young, the MD tissues started to dissolve at the median area of the opisthosoma followed by the tissue at the posterior end, while most of the MD in the anterior part of the opisthosoma remained intact (Fig. 2A–C). During regurgitation, the main body of liquid was concentrated at the posterior rather than the median part of the opisthosoma, while almost no liquid accumulation was observed in the anterior part (Fig. 2D–F). A few days before matriphagy, the anterior, median, and posterior parts of the opisthosoma appeared similar in the level of degradation of the MD lobes (Fig. 2G–I). In all sections, liquid containing nutritional vacuoles was apparent in the perimeter of the section, while the interior contained the remaining MD tissue.

The presence of the lumen of the diverticulum may indicate whether the MD is still functional in the transfer of nutrients through the different stages of maternal care. In sexually mature females, the lumen appeared full of liquid at the median section and empty in the posterior section (Fig. 3A, B). This difference can stem from different ingestion stages of nutrients between the median and posterior sections, but it is clear that the lumen is functional in both areas. The lumen was clearly visible as tissue degeneration began during egg incubation and emergence of the young (Fig. 3C–F). The apparent absence of the lumen in the median section during the egg incubation period may result from the orientation of the section (Fig. 3C), but it was observed after the emergence of the young (Fig. 3E). During the regurgitation stage the lumen was clearly visible in the posterior but not the median section (Fig. 3H), but was not observed before matriphagy in either the median or posterior sections (Fig. 3I, J).

DISCUSSION

Distinct histological changes occurred in the MD of *S. lineatus* females before and during maternal care. The degradation of the MD was gradual, starting after oviposition and ending at matriphagy. However, this degradation process was highly selective; organs, including the ovaries, did not degenerate until after the middle of the regurgitation period. Table 1 summarizes the changes that occurred in different tissues and organs during maternal care.

Prior to maternal care.—In sexually mature females, the MD filled the opisthosoma and surrounded organs such as the heart and ovaries. This finding is in accord with previous work indicating that the MD functions as a nutrient storage site (Nawabi 1974; Ludwig & Alberti 1988, 1990). Indeed our histological sections of sexually mature females show many lipid droplets occurring among and within the MD lobes.

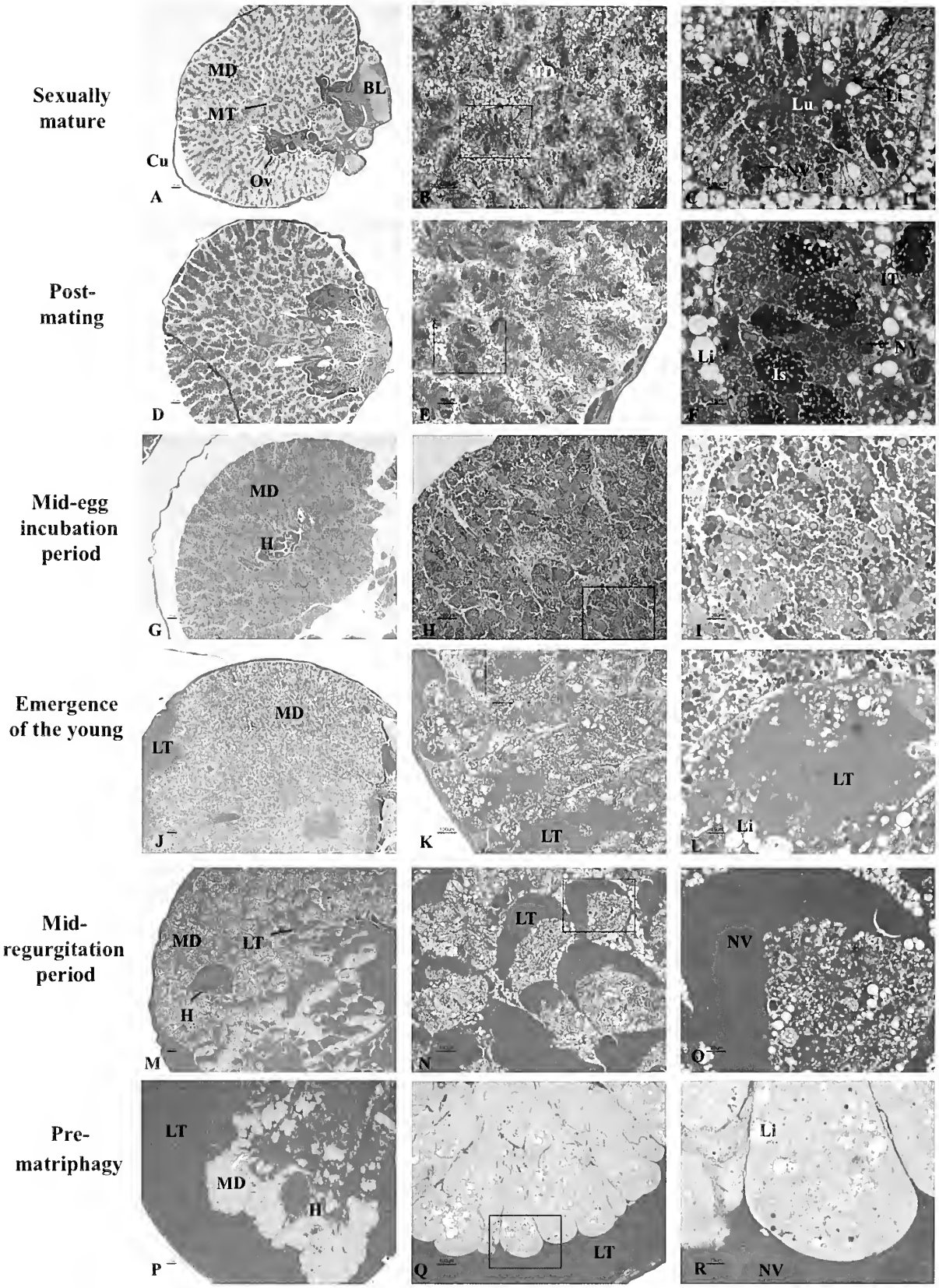

Figure 1.—Light microscopy of median cross-sections in the opisthosoma of *Stegodyphus lineatus* females during reproduction and maternal care. A–C: Sexually mature, virgin female; D–F: Female after mating. Note the dark-blue islets. See text for explanation. G–I: Female during egg incubation; J–L: Female at emergence of the young; M–O: Female halfway through the regurgitation phase; P–R: Female before matriphagy. The magnification of the first image of each stage (left column) is ×2 while the following image (middle column) is ×10. Boxed areas (right column) are enlarged at ×40 magnification. The bar represents 100 μm. Dorsal side facing left. *Cu*: Cuticle; *BL*: Book Lung; *H*: Heart; *Is*: Dark blue islets; *IT*: Intermediate tissue, *Li*: Lipid; *LT*: Liquefied Tissue; *Lu*: Lumen; *MD*: Midgut Diverticula: *MT*: Midgut Tube; *NV*: Nutritional vacuoles; *Ov*: Ovaries.

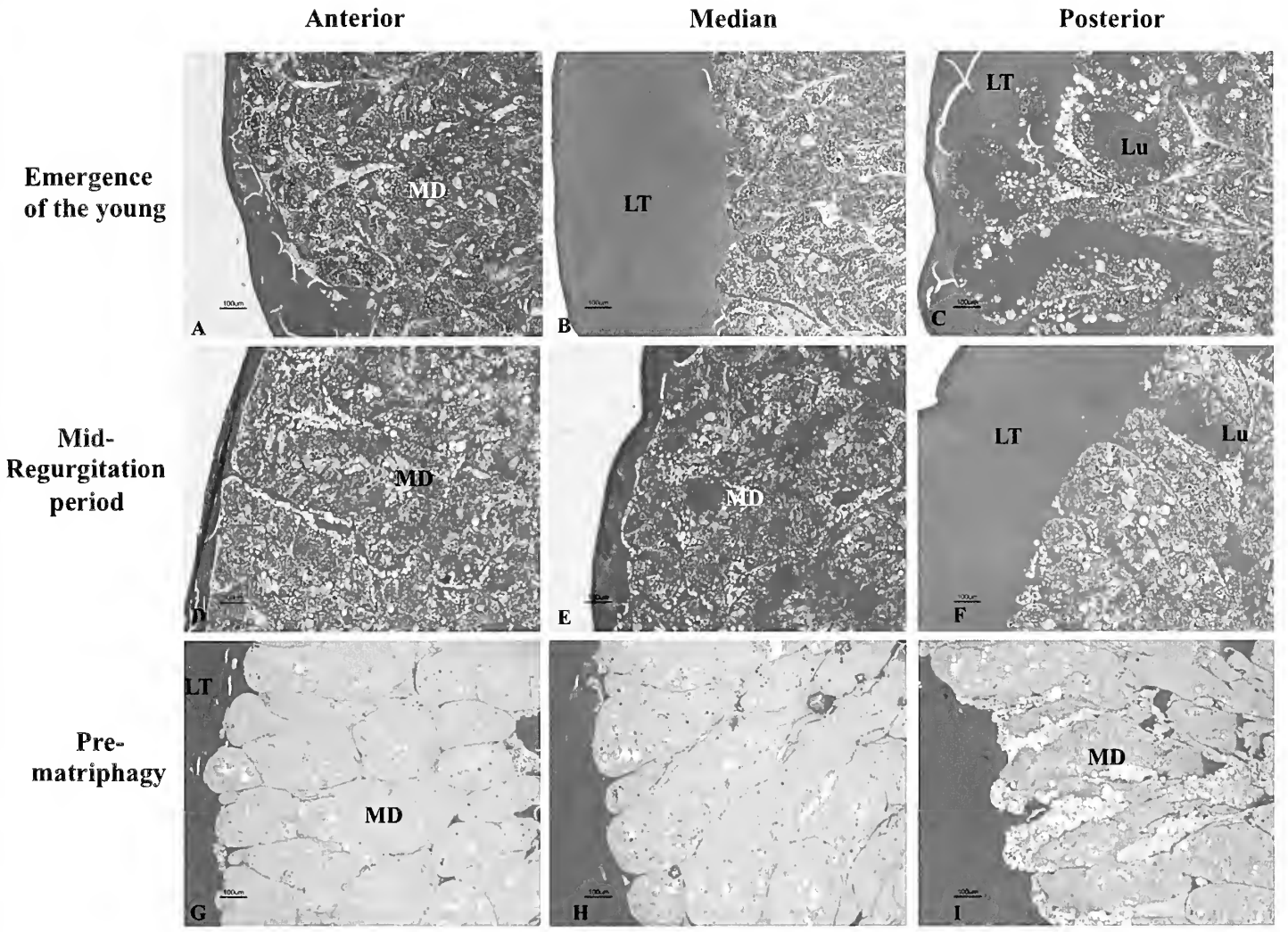

Figure 2.—Light microscopy of cross-sections showing the dorsal side of the opisthosoma of females during maternal care. A–C: Female at emergence of the young; D–F: Female half way through the regurgitation phase; G–I: Female before matriphagy. Images in the left, middle and right columns show anterior, median, and posterior cross-sections, respectively (magnification ×10). The bar represents 100 μm. Dorsal side facing left. *MD*: Midgut Diverticula; *LT*: Liquefied Tissue.

After mating occurs, the structure of the MD remains intact, but the inereased dark-blue (basophilic) staining (dark blue islets) observed may indicate increased presence of ribosomal RNA (di Fiore & Eroschenko 2005). The secretory cells of the MD contain a voluminous, rough endoplasmic reticulum and are characterized by dense granules containing digestive enzymes (Ludwig & Alberti 1990). Thus the dark-blue staining observed in females after mating may indicate increased gene transcription for the production of digestive enzymes (Collatz 1987). We suggest that mating triggers an increased production of digestive enzymes, allowing the female to ingest more nutrients before the young emerge. The timing of these processes corresponds to the period of greatest availability of prey in the spring (Salomon & Lubin unpubl. data). Finally, increased enzymatic activity may be linked as well to the start of the process of MD degradation.

While guarding the egg sac, the female continues to renew her capture web and catch and consume prey (Salomon & Lubin unpubl. data). At this stage the cells and vacuoles are blurred as they start to dissolve within the MD. According to Nawabi (1974), the secretion cells dissolve first, merging their content into the lumen, followed by the digestive cells which deposit their nutritional vacuoles in the intermediate tissue and are then dissolved into the lumen. Consequently, we suggest that nutrients from prey items consumed during the egg incubation period are stored in the intermediate tissue and at a later stage dissolved to constitute the regurgitation fluid accumulating in the lumen together with the degraded secretive and digestive cells in the lumen. The reduced amount of lipid droplets that we observed at this stage may be explained by their having been deposited in the eggs (Laino et al. 2013).

During maternal care.—After the young emerge, the female does not renew her capture web and does not consume prey even when offered under laboratory conditions (Salomon & Lubin unpubl. data). It is not clear from the work of Nawabi (1974) when the digestive cells degenerate, but it seems likely that they absorb nutrients from ingested prey until the emergence of the young. At this stage, we show that the MD lobes in the perimeter of the opisthosoma are

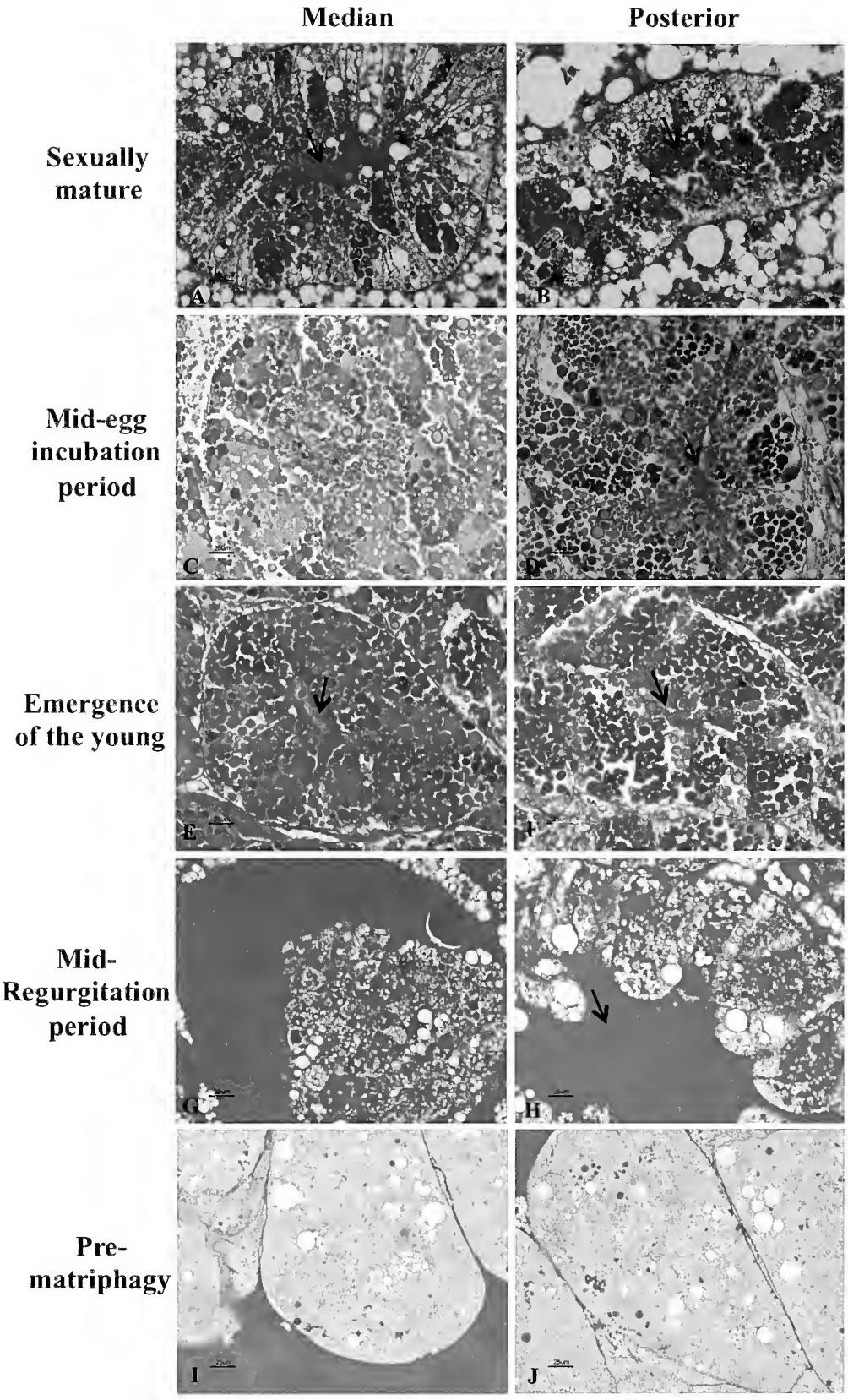

Figure 3.—Light microscopy of cross-sections showing the dorsal side of the opisthosoma of females during maternal care. A, B: Sexually mature female; C, D: Female during egg incubation; E, F: Female at emergence of the young; G, H: Female halfway through the regurgitation phase; I, J: Female before matriphagy. Images on the left are median cross-sections, while those on the right are posterior cross-sections (magnification ×40). The lumen is indicated by a black arrow. The bar represents 100 μm.

Table 1.—A summary of the change of key players in the opisthosoma during maternal care. √ stands for 'present.' Observations of the heart, midgut tube, ovary and MD lumen are not always seen in the representative Figures 1–3.

	Heart	Midgut tube	Ovary	MD- lobe degradation	MD lumen	MD nutritional vacuoles	Lipid droplets	Accumulation of liquefied tissue
Sexually mature	√	√	√	Intact & organized lobes	√	Within the lobe	Within & among the lobes	Not observed
Post-mating	√	√	√	As above	As above	Within the lobe	√	Not observed
Mid-egg incubation period	√	√	√	Blurred boundaries	Barely seen	Within the lobe	Few observed	Not observed
Emergence of the young	√	√	√	Degraded lobes	Not observed	Observed in the liquefied lobe	Observed among the lobes	Observed near the cuticle
Mid-regurgitation period	√	√	Degraded	Degradation intensifies	Not observed	Large vacuoles appear in each lobe	Few observed	Observed near the cuticle & among the lobes
Pre-matriphagy	√	Not observed	Not observed	As above	Not observed	Occur in the liquefied tissue	Few observed	Occupies most of perimeter of the abdomen

dissolved and liquid containing nutritional vacuoles starts to accumulate.

The ovaries are still intact at emergence of the young and even during the regurgitation period (Table 1; not shown), but are no longer visible just before matriphagy. These results are in agreement with a study showing that when all but five spiderlings were removed two days after they emerged, *S. lineatus* females were still able to produce a new clutch of eggs, but females did not produce a new clutch after a similar reduction five or ten days after emergence (Schneider et al. 2003). We suggest that once the female begins regurgitation feeding and tissue degradation intensifies, the window of opportunity for future reproduction closes. Therefore, there is likely a trade-off between current maternal investment, determined by the number of offspring to be fed and future reproductive potential. This trade-off appears strongly skewed towards investment in current reproduction. The reasons for this may be first, regurgitation and matriphagy demand large and possibly irreversible internal changes as shown in this study, and second, the probability of surviving to produce a successful second clutch decreases with time in the season due to ecological pressures such as increased parasite and predator pressure and lower food availability for emerging young (Schneider 1996; Schneider & Lubin 1997b).

By the middle of the period of regurgitation feeding (day 6 after emergence), the young have grown and bodily demand for food increases. This corresponds to increased degeneration of the MD and the appearance of large patches of liquid and nutritional vacuoles. By the 9th day of regurgitation, each young has increased its mass by a factor of 3.8 since emergence (from 0.78 ± 0.11 mg to 2.98 ± 0.28 mg (mean ± SE); Salomon & Lubin unpubl. data) and massive tissue degradation occurs at this time. The large nutritional vacuoles observed may be the result of merging of nutritional vacuoles into large ones or absorption of additional nutrients resulting from degeneration of the tissues. The lipid droplets are not dissolved in this degeneration process and small droplets occur in the MD tissues after emergence of the young, during regurgitation, and even before matriphagy.

Tissue degeneration is unequally distributed within the opisthosoma. At emergence of the young, the tissues are dissolving at the perimeter of the opisthosoma and after 9 days of regurgitation, the interior MD lobes also start to dissolve. Additionally, tissue degeneration starts from the median area followed by the posterior and finally the anterior parts of the opisthosoma. We observed liquid accumulation at the perimeter after emergence of the young, but it was not observed during regurgitation. This remains unexplained. The ovaries, located at the posterior end of the opisthosoma, thus likely remain functional until late in the regurgitation stage.

The presence of the lumen during the egg incubation period indicates that nutrients from ingested prey pass through the lumen. After emergence of the young, the female ceases to consume prey (Salomon & Lubin unpubl. data). The presence of a lumen filled with liquefied tissue at the emergence of the young and during the regurgitation phase confirms that nutrients continue to pass through the lumen. This suggests that nutrients flow from the lumen into the gut and towards the female's mouthparts, constituting the regurgitation fluid that feeds the young. Our data, together with that of Nawabi

(1974), show a gradual process of tissue degradation according to the function of the tissue. First to degenerate are the secretory and digestive cells that are no longer needed to produce digestive fluid or absorb nutrients after the emergence of the young (Nawabi 1974). Next is the intermediate tissue between the MD lobes, leaving a functional MD lumen during the stage of regurgitation. Lastly, the MD lobes degenerate together with the lumen, precluding further regurgitation and setting the stage for matriphagy.

The histological sections show that matriphagy occurs when all the MD tissues are degenerated into a granular non-functional tissue and the perimeter of the opisthosoma is filled with liquid containing numerous large nutritional vacuoles. In *S. lineatus*, matriphagy does not occur earlier than 9 days after emergence of the young (Salomon & Lubin unpubl. data), suggesting that time may be needed to prepare the female's body for matriphagy. Furthermore, after two weeks of regurgitation feeding, the young have grown and molted at least twice (Kullmann 1972). At this stage, their chelicerae are functional and the young have been observed feeding on the opisthosoma and leg joints of the mother (M.S., pers. obs.). The concentration of the nutritional liquid in the perimeter of the opisthosoma allows the young easy access to the liquid food, and as the female's digestive enzymes have already liquefied most of the tissues for the young, they only need to pierce the female's opisthosoma and imbibe the liquid. It remains unknown whether young of *S. lineatus* are capable at this stage of regurgitating digestive enzymes and consuming the remaining MD tissue.

These dramatic changes in the body of female *S. lineatus* are consistent with an extreme semelparous reproductive system in which females invest all of their resources, including their soma, in a single small clutch of offspring (Schneider & Lubin 1997b). This is the first demonstration of the mechanism at the cellular level that underlies suicidal maternal care in an arthropod. In spiders, matriphagy has evolved independently in Amaurobiidae, Eresidae, Theridiidae, and Thomisidae (Pekar 2000; Lubin & Bilde 2007; Yip & Rayor 2013). Similar processes may occur in other taxa exhibiting suicidal maternal care such as the hump earwig *Anechura harmandi* Burr 1904 (Suzuki et al. 2005).

The family Eresidae is an evolutionarily basal family (Johannesen et al. 2007). Maternal care, including regurgitation feeding and matriphagy, occurs in all eresids studied to date (Lubin & Bilde 2007). In the genus *Stegodyphus* Simon 1873, cooperative breeding species have evolved independently three times from subsocial ancestors similar to *S. lineatus* (Johannesen et al. 2007). In colonies of the social spider, *Stegodyphus dumicola* Pocock 1898, non-reproducing females help the mother feed the young through regurgitation and are even consumed by the young (Salomon & Lubin 2007). It is likely therefore that tissue degeneration occurs also in these non-reproductive female helpers. This evolutionary pathway towards cooperative breeding may involve further specialization of females for maternal and allomaternal care of offspring, and at the same time increased dependence of the young on caring adults. Finally, the combination of regurgitation feeding and matriphagy occurs sporadically in other spider lineages (Lubin & Bilde 2007), but whether a similar physiological mechanism underlies this behavior remains unknown.

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Impact of elevated CO_2 on growth, development, and reproduction of the wolf spider, *Pardosa astrigera* (Araneae: Lycosidae)

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Abstract. The effects of elevated CO_2 concentration on spiders were studied using third-instar spiderlings of the wolf spider *Pardosa astrigera* L. Koch 1878 in CO_2 climate chambers with two different concentrations of CO_2 (low, 370 ppm and high, 750 ppm). The food intake and total developmental period of spiderlings reared at high CO_2 concentration increased significantly, and the body length and weight of adult spiders decreased compared to those in the low-CO_2 group. The oviposition rate of female *P. astrigera* and the hatching rate of eggs did not differ between the high- and low-CO_2 groups, but the number of egg sacs and the total number of eggs produced by females from the high-CO_2 group decreased. These results suggest that elevated CO_2 concentrations are harmful to the growth, development, and reproduction of *P. astrigera*.

Keywords: Body length, body weight, oviposition rate

Elevated atmospheric carbon dioxide (CO_2) concentration is thought to be the main reason for global climate change and the greenhouse effect. Climatic records show that the atmospheric CO_2 concentration has increased at an annual rate of 1.9 ppm since 1995. It is estimated that the atmospheric CO_2 concentration will rise to 540–970 ppm by 2100 (IPCC 2013). Because of the increasing atmospheric CO_2 concentration, studying its effects on organisms has been a very popular subject in ecological research (Cannon 1998; Stiling & Cornelissen 2007; Ge et al. 2010; Cornelissen 2011; Foss et al. 2013). Studies to date have mostly investigated the influence of elevated CO_2 on plants, phytophagous insects, and natural enemy insects (predators and parasitoids) (Lincoln et al. 1984; Bezemer & Jones 1998; Wu et al. 2006; Chen et al. 2007; Ge et al. 2010). However, it remains unclear how an elevated CO_2 concentration impacts spiders, a key predator of insects.

Elevated atmospheric CO_2 concentrations have direct effects on the growth, chemistry, and physiology of plants (Lindroth et al. 1993; Roth & Lindroth 1995; Lin & Wang 2002; Veteli et al. 2002; Chen et al. 2005a). For phytophagous and natural enemy insects, elevated CO_2 concentration indirectly affect their growth, development, and reproduction (Brooks & Whittaker 1998; Deng et al. 2002; Chen et al. 2005b, 2007; Wu et al. 2006, 2007; Cornelissen 2011; Foss et al. 2013).

Spiders play important roles in controlling pests (Marc et al. 1999; Barrion et al. 2012) and monitoring environmental conditions (Babczynska et al. 2011; Chen et al. 2011). Understanding the effects of elevated CO_2 concentration on spiders will contribute to better protection and utilization of them. The objective of this study was to determine the effects of elevated CO_2 concentration on spiders. We investigated the food intake, molting, clutch size and egg hatch of the wolf spider *Pardosa astrigera* L. Koch 1878 under different CO_2 concentrations. Based on previous studies about predatory insects (Chen et al. 2007; Ge et al. 2010), we hypothesized that an elevated CO_2 concentration could have either harmful or beneficial effects on the spiders.

METHODS

Spider collection and rearing.—Subadult individuals of *P. astrigera* were collected from farm fields in Ma'anshan Forest Park, Wuhan (30° 52′N, 114° 31′E), Hubei Province, China, in April and May 2012. Voucher specimens were deposited in the Centre for Behavioral Ecology & Evolution, College of Life Sciences, Hubei University, China.

Spiders were kept individually in cylindrical glass tubes (diameter 2 cm, height 12 cm) with a layer of sponge (1.5 cm thick) moistened with water on the bottom. The tubes were plugged with cotton. The spiders were kept in a chamber at 24°C and relative humidity of 60–80% under a light: dark cycle of 14:10 h (lights turned on at 08:00). Every two days, we fed the spiders with adults of *Drosophila melanogaster* cultured under normal CO_2 concentration. Two days post-maturation, females and males were placed together for mating. The male was removed after the female deposited the first egg sac. Following the emergence of second-instar spiderlings and dispersal from the female's abdomen, siblings from different egg sacs were separated and reared individually, with *D. melanogaster* provided as food. Once the second-instar spiderlings molted, they were used for the following experiments.

Spiderling rearing in different CO_2 concentrations.—A total of 300 third-instar spiderlings were randomly selected from different egg sacs and reared individually in cylindrical glass tubes (diameter 2 cm, height 12 cm) and then divided into two groups (150 in each group). The spiderlings were reared under low CO_2 (370 ppm) and high CO_2 (750 ppm) concentrations in CO_2 artificial climate chambers (CC350TLHC type, Changzhou Okefenokee Instrument Co., LTD). The concentrations of CO_2 were set according to Chen et al. (2005b) and Ge et al. (2010); the current concentration of atmospheric CO_2 is 370 ppm, and 750 ppm is approximately double the current level. Each CO_2 concentration was repeated three times by using three chambers, with 50 spiderlings in each chamber. The chambers were set at 25°C and relative humidity of 40%–60% with a light: dark cycle of 14:10 h (lights turned on at

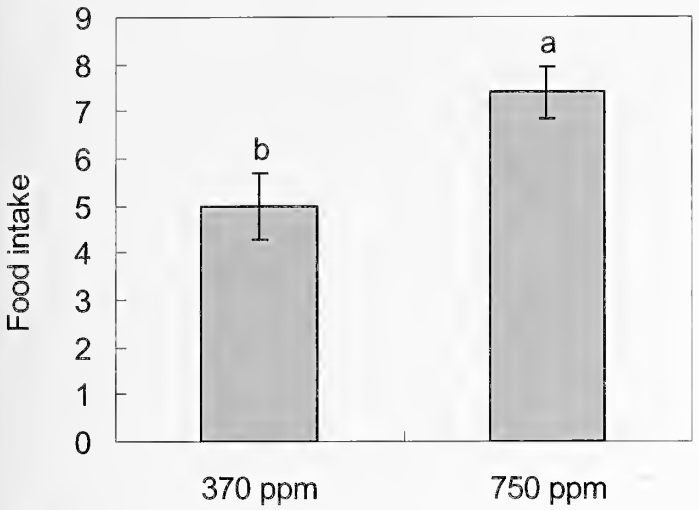

Figure 1.—Effect of different CO_2 concentrations on food intake of *Pardosa astrigera* fed on *Drosophila melanogaster* (n = 10). Different letters above the columns indicate significant differences in food intake of spiders maintained under different CO_2 concentrations (t-test, df = 9, t = 3.39, P = 0.008).

08:00). The spiderlings were fed adults of *D. melanogaster* and the sponges were replaced every two days.

Measurement of food intake of spiderlings.—The food intake of third-instar spiderlings (n = 10) under different CO_2 concentrations was investigated. Fifteen fruit flies (*D. melanogaster*) were provided at 17:00 and the number of living flies was recorded the next day at 09:00. The difference between the initial and final number of flies was taken to be the food intake of the spiderlings.

Assessment of developmental duration, body length, weight, and fecundity.—Molts were recorded when exuviae were observed in the tube, and the time between molts was used as a measure of developmental duration. Two days post-maturation, the females of *P. astrigera* (n = 15) were randomly paired with a mature male and left together for 48 h to mate. Oviposition rate, the number of egg sacs laid by the females (n = 10), the total number of eggs (n = 15), and hatching rate of the eggs (n = 4) were observed and recorded for each treatment (Chen et al. 2011). Ten female and 10 male spiders from different treatments were randomly selected and weighed to the nearest 0.01 mg by using electronic balance (FA1004N type, HANGPING). Then, the above 20 spider individuals were killed in 75% alcohol to measure the body lengths (from front edge of carapace to the end of abdomen) by using an ocular micrometer under a microscope (DFC495 type, LEICA).

Statistical analysis.—Data were expressed as mean ± standard deviation. The differences between treatments were compared by Student's t-test.

Table 1.—Developmental durations (days) of spiderlings of *Pardosa astrigera* under different CO_2 concentrations. Data were expressed as Mean ± standard deviation. The differences between different CO_2 concentrations were compared by Student's t-test. The same notation is used in Tables 2– 4.

Items	CO_2 concentration (ppm) 370	750	t	P
3rd instar	15.4 ± 0.6	20.6 ± 0.9	2.31	0.025
4th instar	13.2 ± 0.5	19.0 ± 0.7	2.29	0.031
5th instar	16.3 ± 0.9	25.1 ± 0.4	2.97	0.008
6th instar	17.1 ± 0.9	22.4 ± 0.4	2.58	0.022
Total	61.8 ± 2.4	87.1 ± 1.5	2.96	0.0037

Table 2.—Body lengths (mm) of adult *Pardosa astrigera* under different CO_2 concentrations (n = 10).

Sex of spider	CO_2 concentration (ppm) 370	750	t	P
Female	9.70 ± 0.84	7.10 ± 0.42	2.66	0.026
Male	8.30 ± 0.45	5.40 ± 0.42	3.14	0.012

RESULTS

The consumption of *D. melanogaster* by the third-instar spiderlings of *P. astrigera* reared at the high CO_2 concentration was significantly higher than at the low CO_2 concentration (t-test, df = 9, t = 3.39, P = 0.008) (Figure 1).

The duration of development (days) for each instar (t-test, $P < 0.05$) and the total duration (t-test, $df1$ = 65, $df2$ = 53, t = 2.96, P = 0.0037) for the spiderlings were significantly longer for the spiderlings kept under high CO_2 concentration than under low CO_2 concentration (Table 1). This suggested that elevated CO_2 caused the spiderlings to mature later, prolonging the total development time.

The body length of female *P. astrigera* was longer than in males kept under the same CO_2 concentration (Table 2). Body lengths of both adult females (t-test, df = 9, t = 2.66, P = 0.026) and males (t-test, df = 9, t = 3.14, P = 0.012) of the high-CO_2 group were shorter than those of the low-CO_2 group. This showed that the body lengths of mature spiders were decreased under the high CO_2 concentration.

The body weight of females of *P. astrigera* was also higher than that of males under the same CO_2 concentration. The body weights of both female (t-test, df = 9, t = 4.78, P = 0.001) and male spiders (t-test, df = 9, t = 2.92, P = 0.017) reared at the low CO_2 concentration were heavier than those reared at high CO_2 (Table 3).

There were no differences in the oviposition rate of female *P. astrigera* (t-test, df = 14, t = 0, P = 1.0) or the hatching rate of eggs (t-test, df = 3, t = 1.01, P = 0.37) between the high- and low-CO_2 groups (Table 4). However, the number of egg sacs produced by the females from the high-CO_2 group was lower than the number produced by the low-CO_2 group (t-test, df = 9, t = 2.69, P = 0.025), and the total number of eggs of the high-CO_2 group was also lower than in the low-CO_2 group (t-test, df = 14, t = 3.49, P = 0.0036).

Table 3.—Body masses (mg) of adult *Pardosa astrigera* under different CO_2 concentrations (n = 10).

Sex of spider	CO_2 concentration (ppm) 370	750	t	P
Female	41.3 ± 8.6	33.5 ± 2.7	4.78	0.001
Male	31.9 ± 0.6	22.4 ± 0.5	2.92	0.017

Table 4.—Fecundity of female *Pardosa astrigera* under different CO_2 concentrations

Items	CO_2 concentration (ppm)		t	P
	370	750		
Oviposition rate (%)	100 ± 0	100 ± 0	0	1.0
Number of egg sacs	5.6 ± 0.9	2.8 ± 0.8	2.69	0.025
Total number of eggs	229.6 ± 39.8	125.3 ± 34.9	3.49	0.0036
Hatching rate (%)	100 ± 0	83.7 ± 15.6	1.01	0.37

DISCUSSION

Under a high concentration of atmospheric CO_2, the nitrogen content of plants decreases, and defoliating insects will increase their feeding rate in order to compensate for the nutritional loss caused by the lower nitrogen content of the host plant (Lincoln et al. 1984). Chen et al. (2007) found that the larvae of lady beetles (*Harmonia axyridis*) consumed more cotton aphids *Aphis gossypii* fed on cotton plants grown in elevated CO_2 in order to compensate for the reduced soluble protein in *A. gossypii* owing to the decrease in foliar N and the increase in the C:N ratio in the cotton plants. Our results showed that the third-instar spiderlings of *P. astrigera* also consumed more fruit flies at the high than at the low CO_2 concentration. This phenomenon may have been caused by the larger amounts of energy required by the spiders and higher respiration rates (Foss et al. 2013) to compensate for their increased activity in the high-CO_2 environment.

High atmospheric CO_2 concentration could affect enzyme activity in the cotton bollworm *Helicoverpa armigera*, and the available nutrients would decline significantly, thereby impairing the growth and development of *H. armigera* (Chen et al. 2005b). The body length and weight similarly decreased in *H. armigera* (Chen et al. 2005b). When second-stadium gypsy moth (*Lymantria dispar*) larvae were grown under high CO_2 concentration, their body weight was lower than in the normal CO_2 concentration (Wang et al. 2006). Our former results showed that when *P. astrigera* was subjected to the stress of Pb or Zn, its developmental duration lengthened (Chen et al. 2011). Similar results were found in our present study. The development time of *P. astrigera* lengthened and the body length and weight of mature spiders decreased. This suggests that these different environmental factors can cause developmental delay in this spider species.

Our results indicated that the fecundity of *P. astrigera* was significantly decreased at the high CO_2 concentration. Brooks & Whittaker (1998) found that the number of eggs produced by females of the green dock beetle *Gastrophysa viridula* that were continuously cultured for three generations, decreased under a high atmospheric CO_2 concentration. At the high atmospheric CO_2 concentration, the number of eggs laid by females of *H. armigera* was markedly reduced (Chen et al. 2005b). However, some studies have had different outcomes. Bezemer & Jones (1998) showed that the number of eggs laid by females of the winter moth *Operophtera brumata* could increase, and the number of eggs laid by *G. viridula* did not differ significantly between high and normal CO_2 concentrations (Brooks & Whittaker 1998). In the present study, female spiders laid fewer eggs under the stress of high CO_2 concentration. Taken together, these studies show that the impact of elevated CO_2 on the reproduction of arthropods varies among species.

In summary, the high CO_2 concentration proved to be harmful to the growth, development, and reproduction of *P. astrigera*. The results of this experiment increase understanding of the responses of spiders reared in elevated atmospheric CO_2 concentration.

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Alien spiders in Chile: evaluating Darwin's naturalization hypothesis

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Abstract. Darwin's naturalization hypothesis (DNH) states that the successful establishment of alien species is favored when the phylogenetic relationship between the colonizer and the recipient community is distant. From a population perspective, the establishment involves both the progressive increase in size and spatial distribution of the invasive population. In this study, we focused our attention on the spatial component of establishment, assessing the role of phylogenetic relatedness as a determinant of its extension. Following DNH, it is expected that alien species closely related to the native spiders would show narrower distribution ranges than alien taxa less related to the native species. We found 18 alien spider species in Chile; all of these are synanthropic and most are of African origin. Our results indicate a difference in range size between related and unrelated species but it was not statistically significant. Consequently, the results do not support DNH as an explanation of the distributional component of establishment of alien spider species in Chile. We conclude that ecological constraints do not affect the process of invasion of spiders; therefore, it is only time that determines the spread of alien spiders in this country.

Keywords: Aerial dispersal, biological invasion, geographic range, residence time, phylogenetic relatedness

Establishment of species beyond their natural range is on the rise because of increasing trade, transport, travel and tourism that are part of globalization. This provides living plants, animals and biological materials with vectors and pathways crossing the biogeographical barriers that would usually block their way (Shine et al. 2009).

Currently, organisms belonging to different taxonomic groups are translocated from one region to another, with which they do not share a prior history (Williamson 1996; Davis 2009). Although it is estimated that most of the organisms that disperse do not successfully establish in the target area, sometimes a small number of propagules can configure a founder colony and become established (Kolar & Lodge 2001; Sakai et al. 2001). One of the central challenges in the study of biological invasions has been to understand what factors determine this establishment process (Williamson 1996; Lockwood et al. 2007; Davis 2009), understood as a population expansion event.

Several hypotheses have been proposed to explain why some species are able to establish and others are not (Kolar & Lodge 2001; Mitchell et al. 2006). A particularly intriguing theory has been called Darwin's naturalization hypothesis (DNH) (Daehler 2001). This hypothesis, originally proposed by Darwin (1859), assumes that establishment success is influenced by the phylogenetic relationship between the colonizer and the members of the receiving community (Chesson 2000; Adler et al. 2007). In this context, invasive species that exhibit close phylogenetic relationships with the recipient community should display a high niche overlap, thus generating a high intensity of competition with members of the receiving community (Cahill et al. 2008; Cavender-Bares et al. 2009; Mayfield & Levine 2010). Therefore, under conditions of greater phylogenetic relationship to the recipient community, a colonizer would less likely establish oneself. Inversely, when the colonizer has a low level of relationship with members of the community, DNH suggests that competitive intensity decreases, a fact that will help to facilitate the establishment of invasive species. Although there are various concepts of establishment (Richardson et al. 2000), from the population point of view, establishment can be visualized as a process in which a colony of alien species, once introduced, independent of intentional human assistance, has its population increase in size and expands into an area of colonization (Sakai et al. 2001; Shigesada & Kawasaki 2001). Thus, depending on the abundance and distribution levels, it is possible to recognize various stages of progress in the establishment process (Shigesada & Kawasaki 2001). Therefore, if the phylogenetic relationship determines the distribution component, as predicted by DNH, alien species more related to members of the community would show smaller distributional ranges when compared to those of less related taxa.

Generalist arthropod predators include invasive species that are capable of affecting native species through a variety of direct and indirect pathways (Snyder & Evans 2006). Invasive generalist arthropod predators can displace native predators primarily through competition, intraguild predation, transmission of disease, and escape from predation and/or parasites (Snyder et al. 2004). As generalist arthropod predators, spiders have the potential to affect native arthropod species assemblages; nevertheless, spiders have been largely overlooked as invasive species (but see Nyffeler et al. 1986; Hann 1990; Gruner 2005). Once established, invasive spiders may be viewed as either beneficial arthropods in agroecosystems, or as keystone predators in native ecosystems. Documented displacements of native spiders by invasive spider species are rare, although Nyfeller et al. (1986) and Hann (1990) reported cases of competitive exclusion between invasive and native spider species in Europe and New Zealand, respectively.

Because distributional range is one of the components of the establishment process, the objective of this study is to evaluate DNH and its effect on the distributional range of alien spiders in continental Chile. For this, we characterized the distributional ranges of alien spiders that differ in phylogenetic relatedness to native spiders, while also considering other

possible factors that can affect range size such as the minimum residence time (i.e., the time since the introduction of a species to a region) and aerial dispersal or ballooning (Thebaud & Simberloff 2001; Bell et al. 2005).

METHODS

Spider data and distributional range.—We used the World Spider Catalog (2014) for assigning the distribution of species in the world; also we eomplemented these data with an intensive literature survey and collections in different cities of Chile to determine the distribution in the national territory. We considered alien species that are not native to the country and/or have a cosmopolitan distribution in the world today, where the current distribution already refleets human influence. Single reeords of non-established spider species and doubtful records were excluded. From this information, the total number of administrative regions occupied by each species was established and then the latitudinal extension (in kilometers) was determined. For this purpose, the distributional range was estimated as the sum of the maximum length of each oceupied administrative region (Instituto Geografico Militar 2010); this procedure assumes that each species is distributed throughout each region (see Castro et al. 2005).

The phylogenetic relatedness between the alien spiders and resident community was classified in three levels. The first level (Close group) was used for species belonging to a genus that is represented in the native fauna of Chile. The second level (Intermediate group) was used for species belonging to genera not represented in the native spider fauna but from a family present in the native fauna. Finally, the third and most distant level (Distant group) was used for species belonging to genera and families not represented in the native spiders.

Because the area of origin is quite often not well known, the most probable origin of these spiders was taken from Kobelt & Nentwig (2008). The alien spider origins were attributed to the following five categories: a) Africa, b) Asia, c) Europe, d) America (refers to the tropical part of America) and e) "unknown" when the origin of some alien spider species is not exactly known but the species is globally distributed.

The minimum residence time was obtained from the oldest known record of the species obtained from historical information and collections. In addition, a measure of aerial dispersal, or ballooning, was included at an ordinal scale of 0 = not known, 1 = present, based on Bell et al. (2005).

Statistical analyses.—We performed a Generalized Linear Model (GLM) with normal error structure and the identity link function using STATISTICA 6.0 program (Stat Soft 1999) for analyzing simultaneously the effect of categorical (dispersal mode, phylogenetic relatedness) and continuous (residence time) variables on the dependent variable (distribution range, in km). The normality of residuals was analyzed with a Kolmogorov-Smirnov test after fitting the model. Phylogenetic relatedness was used to test DNH where dispersal mode and residence time were tested for other ecological factors that can explain distribution range.

RESULTS

Taxonomy of alien species and distribution in Chile.—We found 18 alien species belonging to 11 families. All alien spiders in this study are synanthropic species with 13 being considered cosmopolitan (Table 1), where Theridiidae were represented by four species, the most abundant family. The families Araneidae, Agelenidae, Pholcidae, and Salticidae were represented by two species each. Six other families are represented by only one species. The most astonishing aspect of the composition of the alien spider fauna is that it does not reflect the structure of the Chilean spider fauna. Only eight of the 11 families of alien spiders are also present in Chile. The families Agelenidae, Oecobiidae and Dysderidae are not represented in the native fauna (Fig.1). Many of the spiders are of African origin (33.3%), followed by European (27.7%) and Asian species (22.2%) and finally South American species (5.5%), however, the biogeographical origin of the other species is unknown. The species of European origin are those with the highest average distributional range in Chile (2081 km), followed by the species of Asian origin (1738 km) (Table 2).

The regions with the highest number of alien species are the Tarapaca region (11 species) and the Antofagasta region (10 species) in northern Chile (Fig. 2). The spiders *Pholcus phalangioides* (Fuesslin 1775) (Pholcidae) and *Steatoda grossa* (C. L. Koch 1838) (Theridiidae) are the most widely distributed in Chile, being found from Arica (18° 28′ S, 70° 52″ W) to Magallanes (53° 9′ S, 70° 55′ W). Other species widely distributed in Chile are *Dysdera crocata* C. L. Koch 1838 (Dysderidae), *Tegenaria domestica* (Clerck 1757) (Agelenidae), *Menemerus semilimbatus* (Hahn 1827) (Salticidae), *Urozelotes rusticus* (L. Koch 1872) (Gnaphosidae) and *Oecobius navus* Blackwall 1859 (Oecobiidae). The species *Hasarius adansoni* (Audouin 1826) (Salticidae), *Latrodectus geometricus* C. L. Koch 1841 (Theridiidae), *Heteropoda venatoria* (Linnaeus 1767) (Sparassidae) and *Smeringopus pallidus* (Blackwall 1858) (Pholcidae) are found exclusively in the north of Chile (Table 1).

Minimum residence time.—Only three species had a minimum residence time greater than 100 years. Most species have a residence time between 1 and 24 years (Table 2). GLM analysis showed a significant positive effect of the minimum residence time on the size of the distributional range (Fig. 3) (GLM, $F_{1, 11} = 37.7$, $P < 0.05$). Spiders with longer residence time in the country include *P. phalangioides*, *T. domestica*, *S. grossa*, *D. crocata* and *U. rusticus* (Table 1).

Darwin's naturalization hypothesis and distributional range.—Twenty eight per cent of alien species belong to a phylogenetically related group. Exactly half of the species belong to families already represented in the native fauna (Intermediate group); while the distant group represented only 22% of the alien species. The set of species with higher levels of phylogenetic relationship (Close group) with native fauna (i.e., congeneric species) had an averaged distribution range of 1452.2 km, while those distant species (Distant group) showed the greatest geographical extension (1710 km), but these differences were not statistically significant (GLM, $F_{2,11} = 0.4$, $P = 0.65$). The aerial dispersal did not affect the distribution range (GLM, $F_{1, 11} = 0.9$, $P = 0.35$).

DISCUSSION

We can say that the effect of residence time can be interpreted as a neutral hypothesis; only time of arrival of alien spiders is enough to predict invasiveness. If this is true,

Table 1.—List of alien species, their earliest records, biogeographical distribution, and distribution in Chile. Area of origin: since the area of origin is quite often not well known, this refers to the most probable origin.

Taxa	Area of Origin	Geographic distribution	First record in Chile	Distribution in Chile
Araneidae				
Argiope trifasciata	Africa	Cosmopolitan	Levi (1968)	From Salamanca to Lanquihue
Zygiella x-notata	Unknown	Cosmopolitan	Mello-Leitão (1951)	Very common in southern Chile, Santiago to Los Lagos Region.
Agelenidae				
Tegenaria domestica	Europe	Cosmopolitan	Simon (1904)	Center of Chile to Magallanes region
Tegenaria pagana	Europe	Europe to Central Asia, USA to Chile, New Zealand	Roth (1968)	Center of Chile: Metropolitan and Valparaiso region
Dysderidae				
Dysdera crocata	Europe	Cosmopolitan	Nicolet (1849)	From Antofagasta to Bio-BioRegion
Oecobidae				
Oecobius navus	Africa	Cosmopolitan	Santos & Gonzaga (2003)	North and center of Chile : From Iquique to Bio-Bio Region
Pholcidae				
Pholcus phalangioides	Asia	Cosmopolitan	Nicolet (1849)	From Arica to Magallanes Region
Smeringopus pallidus	Africa	Pantropical	Taucare-Ríos (2012)	North of Chile: Tarapaca Region
Prodidomidae				
Prodidomus rufus	Unknown	Cosmopolitan	Platnick & Baehr (2006)	Antofagasta Region
Gnaphosidae				
Urozelotes rusticus	Asia	Cosmopolitan	Simon (1904)	Atacama to Valparaiso Region
Salticidae				
Hasarius adansoni	Africa	Cosmopolitan	Taucare-Ríos (2013b)	Arica and Parinacota and Tarapaca Region
Menemerus semilimbatus	Africa	Canary Islands, southern Europe, western Asia, and Africa; and introduced to Argentina, Chile, and USA.	Taucare-Ríos & Edwards (2012)	Arica and Parinacota to Maule Region
Scytodidae				
Scytodes univittata	Asia	Canary Is. to Myanmar, synanthropic in Neotropics	Brescovit & Rheims (2000)	From Arica to Chañaral
Sparassidae				
Heteropoda venatoria	Asia	Pantropical	Taucare-Ríos & Brescovit (2011)	Tarapaca Region: Iquique
Theridiidae				
Latrodectus geometricus	Africa	Cosmopolitan	Taucare-Ríos (2011)	From Arica to Mejillones
Parasteatoda tepidariorum	South America	Cosmopolitan	Levi (1967)	North to center of Chile: Antofagasta to Santiago
Steatoda grossa	Europe	Cosmopolitan	Simon (1904)	From Arica to Magallanes
Steatoda triangulosa	Europe	Cosmopolitan	Taucare-Ríos et al. 2013	Tarapaca Region

then the effect of other ecological variables is not significant. We failed to prove that the aerial dispersal and the interspecific competition between phylogenetically related species (DNH) would play a significant role in the invasion success of the alien spiders in Chile. The distributional range increased in size as residence time in the invaded region increased.

The number of alien species represents about 2% of known spiders in Chile. The family Theridiidae includes the largest number of alien species, agreeing with the results obtained by Kobelt & Nentwig (2008). Apparently the species of this family have a predisposition to be alien due to their link with human dwellings (Kobelt & Nentwig 2008). Globally common families, such as Tetragnathidae, Lycosidae and Zodariidae, are not represented at all among the alien species in Chile, probably because some families are usually not associated with human infrastructure and have a rather low probability of becoming transported to foreign areas (Kobelt & Nentwig

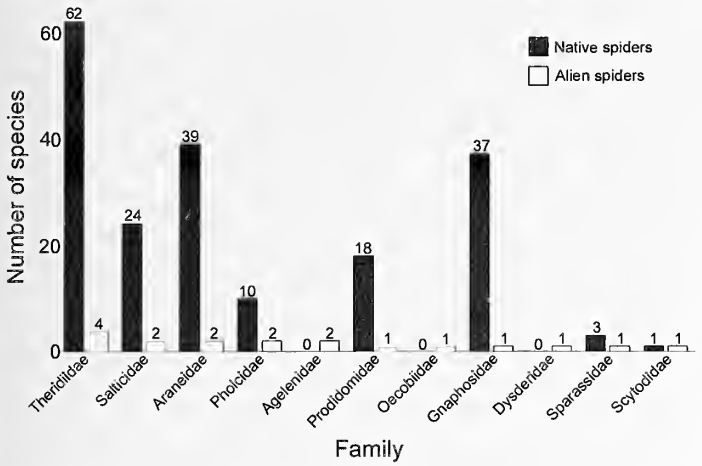

Figure 1.—Taxonomic overview of alien spider species in Chile compared to the native Chilean fauna. Families are presented in decreasing order based on the number of alien species.

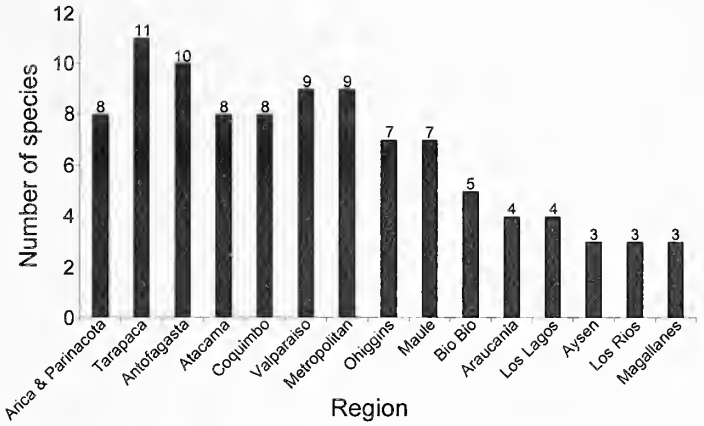

Figure 2.—Number of alien spider species for each region of Chile. Arranged from north to south.

2008). Most alien spiders are of African origin, which is consistent with the results obtained by Kobelt & Nentwig (2008), and currently have cosmopolitan distributions (*Argiope trifasciata* (Forsskål 1775), *H. adansoni, O. navus* and *L. geometricus*) (Levi 1968; World Spider Catalog 2014). However, the species with the highest distributional range were the spiders of European origin, namely: *D. crocata*, *S. grossa* and *T. domestica* (Simon 1904; Roth 1968; Levi 1967; Ramirez et al. 2004; Taucare-Rios et al. 2013) (Table 2). A comparison between temperate and tropical origins indicates that about 30% of the species originate from temperate habitats (Europe) and about 60% are from the tropical habitats (Asia, Africa and America); the climate habitats of the others are unknown. Uncertainty, however, is high because for many species very little is known about the natural environment in which they live in the area of origin.

Residence time is a critical variable to predict invasiveness (Wilson et al. 2007), a fact that is reinforced by our results. The species with the highest residence time in Chile were *P. phalangioides* and *D. crocata*, described and reported for the first time in Chile over 160 years ago by Nicolet (1849). Similarly *Urozelotes rusticus*, *Tegenaria domestica* and *Steatoda grossa*, reported by Simon (1904), had a minimum residence time of about 110 years. To date, these species have significantly expanded their distribution in the country invading from the arid climate of northern Chile through the humid and cold climates in south Chile (Simon 1904; Cekalovic 1976; Platnick & Murphy 1984; Taucare-Rios 2010; Taucare-Rios et al. 2013). Other species reported in Chile have a wide distribution. These species include *M. semilimbatus*, *Scytodes univitatta* Simon 1882, *Zygiella x-notata* (Clerck 1757) and *O. navus* (Mello-Leitão 1951; Levi 1974; Brescovit & Rheims 2000; Santos & Gonzaga 2003;

Table 2.—Size of the geographic range (mean ± SD) for 18 species of alien spiders inhabiting continental Chile. This information is organized according to the minimum residence time, aerial dispersal, biogeographic origin and the phylogenetic relatedness.

Factors	Size [km]	N
Minimum residence time		
160 years	2868.5 ± 1416.3	2
110 years	2574.6 ± 1144.2	3
63 years	1309 ± 0.0	1
47 years	1317.6 ± 17.5	3
22 years	1441.0 ± 0.0	1
14 years	1257.0 ± 0.0	1
8 years	378.0 ± 0.0	1
3 years	422.0 ± 384.0	2
2 years	779.2 ± 612.5	4
Biogeographic origin		
Asia	1738.2 ± 1557.2	4
Europe	2081.0 ± 631.9	5
Africa	963.1 ± 631.9	6
Unknown	614.0 ± 333.7	2
America	1318.0 ± 0.0	1
Aerial dispersal		
Not known	1113.8 ± 701.0	8
Present	1618.3 ± 1331.8	10
Phylogenetic relatedness		
Close group	1452.2 ± 1425.6	5
Intermediate group	1226.1 ± 1176.9	9
Distant group	1710.0 ± 397.8	4

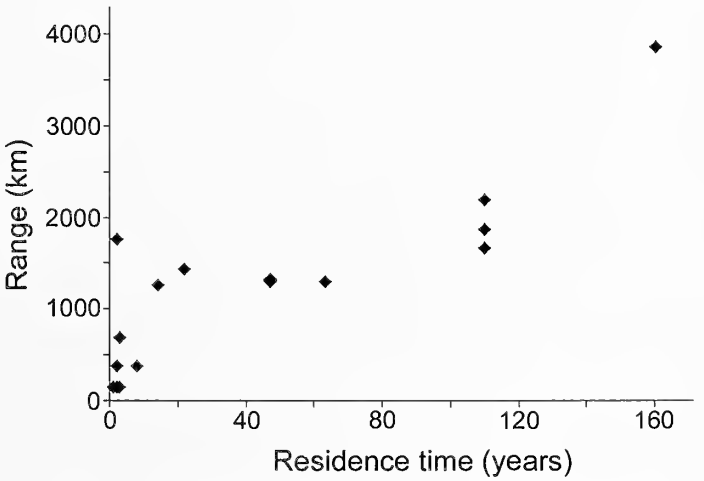

Figure 3.—Relationship between residence time and distributional range of alien spider species.

Taucare-Rios & Edwards 2012; Taucare-Rios 2013a; Taucare-Rios et al. 2013). Finally, species reported recently, such as *L. geometricus*, *S. pallidus, H. adansoni, Prodidomus rufus* Hentz 1847, and *H. venatoria*, have a limited distribution in Chile (Platnick & Baehr 2006; Taucare-Rios & Brescovit 2011; Taucare-Rios 2011, 2012, 2013b); these results show a clear correlation between the minimum residence time and distributional range of the species. Other studies have also suggested a positive relationship between residence time and current distribution of alien species (Rejmánek 2000; Castro et al. 2005; Hamilton et al. 2005; Wilson et al. 2007).

Shipping traffic is responsible for the majority of accidental generalist arthropod predator introductions (Snyder et al. 2004). For example, the ground beetle *Pterostichus melanarius* Illiger 1798, a European native that has invaded a large part of North America, is believed to have arrived in soil ballast dumped from ships (Niemela et al. 1997). Also it is known that potted plants and container shipments with manufactured goods are important modes of introduction for alien arachnids (Kobelt & Nentwig 2008; Nentwig & Kobelt 2010). In Chile, most of the alien species are present in coastal regions (e.g., Tarapaca, Antofagasta, and Valparaiso Regions), where they probably arrived because of commerce and the relocation and travel of people; and from there they were transported by humans to other localities. It is known that some alien spider species may have been introduced to the northern ports (Taucare-Rios 2011; Taucare-Rios & Brescovit 2011; Taucare-Rios & Edwards 2012), ineluding pantropical and cosmopolitan species from Asia and Africa, such as *M. semilimbatus*, *H. venatoria*, *P. phalangioides*, *S. pallidus* and *L. geometricus*.

The modes of range expansion in spiders vary. For example, small web-building spiders naturally spread by means of ballooning. By this means, they can be transported hundreds of kilometers with the help of air currents (Bell et al. 2005). However, ballooning is not regularly used by large invasive species (Walter et al. 2005). Such species and non-ballooning species must have used other means, in particular human mediated transfer (cf. Rabitsch 2011). Thus, the contribution of aerial dispersal in the alien spiders in Chile is less important compared to the facilitated transport by humans.

Finally, we can say that our results do not support DNH as a plausible explanation of the distributional component of establishment of alien spiders in Chile. This hypothesis arose from a related hypothesis of Darwin (1859) that closely related species tend to possess similar niches and, hence, perform similarly under the same environmental conditions (for a recent empirical example, see Brandt et al. 2009), translating into strong competition imposed by resident species on closely related invaders that reduces their success. Within this context, there have been multiple attempts at testing DNH (reviewed in Proches et al. 2008). Together, these studies have reported positive (Daehler 2001; Duncan & Williams 2002), negative (Rejmanek & Richardson 1996; Strauss et al. 2006), or no (Lambdon & Hulme 2006; Ricciardi & Mottiar 2006) agreement with DNH. However, DNH best applies to small spatial scales at which species interact with each other. Given the assumption of strong competition between closely related species as a driving mechanism (Proches et al. 2008), it does not always happen, since competition may not be relevant to some alien species.

Our results show that all alien spider species in the country are synanthropic and may not compete strongly with native species which do not usually inhabit urban environments, therefore, maybe competition is not relevant in the establishment of the alien spiders in Chile. Others factors could be more important for these animals in anthropogenic environments. For example, it is known that on a geographic level, macro-environmental conditions (climate, precipitation, temperature, etc.) do influence the size of a species' range, even more than do interspecific interactions (see also Chesson 2000; Hubbell 2001; McKinney 2006; Sax et al. 2007).

This work summarizes knowledge about the alien spiders in Chile and the ecological process that may determine their establishment. In this context, our results do not support DNH, but do show the importance of minimum residence time for the establishment process of the alien spiders in Chile. Early warning plans will be very efficient to control the invasion of alien spiders, beeause in the absence of ecological constraints, the success of the invasion might be greater. However, future studies may shed light on other ecological processes involved in the successful invasion of these arthropods in Chile, mainly linked to the influence of human activity and possible events of facilitation in this country.

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A re-evaluation of the formula to estimate the volume of orb web glue droplets

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Abstract. The size and shape of the glue droplets along the spiral threads of orb webs play an important role in web function. Despite this, methods for estimating droplet volume are not well defined, with contradicting formulas published. Here we address the discrepancies in the published formulas with a mathematical derivation that assumes that a glue droplet conforms to a parabola along one side of the axial line. We confirmed the validity of our derived formula by comparing it with the results of numerical integration. We also document that a droplet continues to conform to a parabola as its volume changes with environmental humidity. Our formula can be applied simply by colleeting the spiral threads, examining the droplets under a light microscope and measuring their length and width, making it easy to compare the droplets of different species collected at different relative humidities.

Keywords: Aggregate glue, computer simulation, mathematical proof, spiral threads

The volume, shape, number and chemical composition of glue droplets on orb web spiral threads have important consequences for the adhesive properties of orb webs (Opell 2002; Opell & Hendricks 2007; Opell et al. 2011b, 2013; Sahni et al. 2011, 2014; Torres et al. 2013). As such, many recent studies have estimated glue droplet volume to ascertain how orb web spirals might function across a range of scales (e.g., Opell & Hendricks 2007, 2009, 2010, Opell & Schwend 2007, 2009; Wu et al. 2013; Blamires et al. 2014).

The glue of orb webs is spun fully coating the underlying pair of flagelliform fibers. The compounds in the glue render it hygroscopic and, as a consequence, the glue readily absorbs atmospheric moisture (Edmonds & Vollrath 1992; Higgins et al. 2001; Sahni et al. 2011; Stellwagen et al. 2014). A combination of internal forces and surface tension creates Rayleigh instability so the glue forms into droplets (Vollrath & Edmonds 1989, 2013; Edmonds & Vollrath, 1992; Opell et al. 2013); the same forces that make water in air form into droplets. Under a light microscope, the droplets appear parabolic and periodically positioned along the flagelliform fibers resembling "beads along a string" (Sahni et al. 2012; Torres et al. 2013; Wu et al. 2013).

Estimating the volume of the glue droplets is generally done by collecting samples of spiral silk from webs, examining them under a light microscope at 100×–1000× magnification, and measuring the length (l) of the droplet along the capture thread's paired axial lines and the width (w) of the droplet perpendicular to the axial lines. The volume, V, of an individual orb web spiral droplet has, in most cases, been estimated from the formula:

$$V = \frac{2\pi(w)^2 l}{15} \quad (1)$$

(Opell & Schwend 2007, 2009; Agnarsson & Blackledge 2009; Opell & Hendricks 2009, 2010; Wu et al. 2013; Stellwagen et al. 2014). This formula is a corrected version of a formula first used to determine the percentage of material invested in smaller secondary droplets of viscous threads (Opell & Hendricks 2007) and was reported as:

$$V = \frac{2\pi(h)^2 b}{15} \quad (2)$$

where the values h and b appear to have been incorrectly defined as "h = 0.5 droplet width and b = 0.5 droplet length". This paper therefore: (i) formally addresses the discrepancy in the formulas used to compute droplet volume, (ii) provides the first detailed mathematical derivation of the corrected formula, and (iii) confirms the validity of the corrected formula using a computer simulation.

Furthermore, the compounds in the glue from orb-web viscous threads confers hygroscopicity, thus droplet volume changes with environmental humidity (Townley et al. 2006; Opell et al. 2011a; Opell et al. 2013; Townley & Tillinghast 2013). We thus confirmed the applicability of our formula for droplet volume computation under different conditions by determining whether droplet shape remained a parabola over a wide range of humidities.

FORMULA REEXAMINATION

We derived a droplet volume formula based on the conclusion that the profile of a droplet on one side of the axial line assumed the configuration of a parabola and that droplet volume can, therefore, be computed as the volume encompassed by a parabola rotated around this axis. The requirement that a droplet's profile be a parabola was confirmed for droplets of *Leucauge venusta* (Walckenaer 1841), *Metepeira labyrinthea* (Hentz 1847), *Aranens pegnia* (Walckenaer 1841), and *Aranens marmoreus* Clerck 1757. For these species the droplets' regressions of coordinates predicted by a parabola against those measured on droplet images had $R^2 > 0.97$ (Opell & Hendricks 2007). Therefore, each quadrant of a droplet represents a parabola (Fig. 1) whose coordinates are expressed as:

$$y = h(1-(x/b)^2),\ 0 \le x \le b \quad (3)$$

where $b = l/2$ and $h = w/2$ (Opell & Hendricks 2007).

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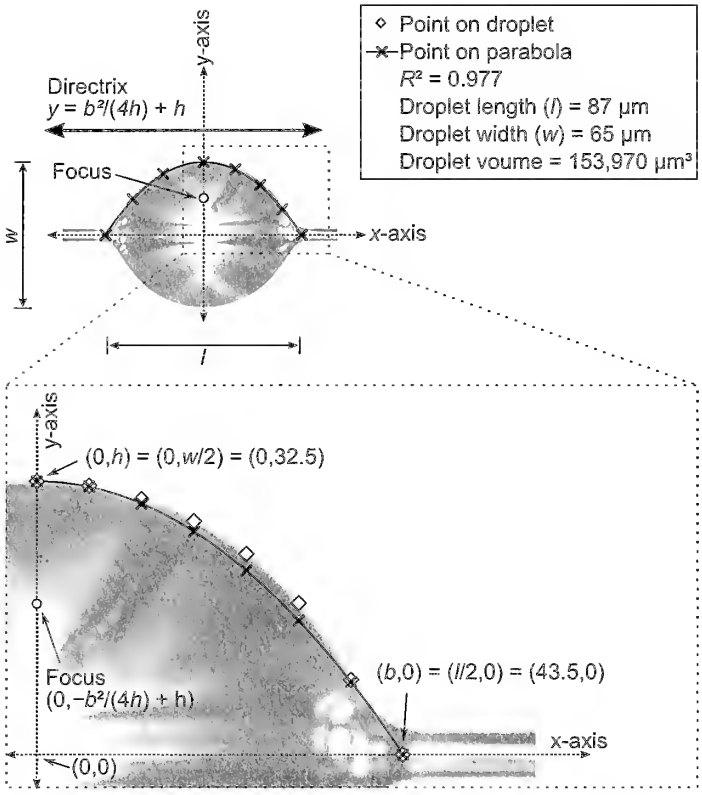

Figure 1.—Viscous thread droplet of *Araneus marmoreus* photographed at 90% relative humidity, showing fit of coordinates to a parabola, reference points used in volume formula computation, and the volume computed for this droplet.

Accordingly, droplet volume is calculated by rotating y around the x-axis and multiplying this value by 2, so

$$V = \frac{16\pi h^2 b}{15} \tag{4}$$

Substituting $w = 2h$ and $l = 2b$, we obtain formula (1), which allows the measured dimensions of droplets to be used directly in computing droplet volume. Details of the derivation of equation (4) follow.

A parabola is defined as the points equidistant between a line, called the directrix, and a point, called the focus. To derive the equation for the parabola that approximates the outline of a droplet on the axial line pair of an orb-weaving spider's web, we placed the axial lines on the x-axis and the widest part of the droplet on the y-axis. We assigned the length of the droplet along the x-axis as l and the width of the droplet along the y-axis as w; see Fig. 1. We let $b = l/2$ and $h = w/2$. The right-hand endpoint of the droplet on the x-axis is $(b, 0)$. The top point of the droplet on the y-axis is $(0, h)$, and we call the distance between this point and the directrix and between this point and the focus $p/2$.

The focus is the point $(0, -((p/2) - h))$, which equals $(0, (h - (p/2)))$; the equation for the distance to the directrix at point $(b, 0)$ is

$$y = h + (p/2) \tag{5}$$

and the distance to the focus is

$$y = sqrt\,(b^2 + (h - (p/2))^2) = sqrt\,(b^2 + (p^2/4) - p\,h + h^2). \tag{6}$$

Since the distances (5) and (6) are equal in a parabola, we equate these equations, square both sides, and simplify:

$$\begin{aligned} (h + (p/2))^2 &= b^2 + (p^2/4) - p\,h + h^2 \\ p/2 &= b^2/4h \end{aligned} \tag{7}$$

The distance between point (x,y) on the parabola and the directrix is its distance to point $(x, (p/2) + h)$ on the directrix:

$$sqrt\,(y - ((p/2) + h))^2. \tag{8}$$

The distance between point (x,y) on the parabola and the focus point $(0, (h - (p/2)))$ is

$$sqrt\,(x^2 + (y - h + (p/2)^2)). \tag{9}$$

If we substitute equation (7) for p/2 and then equate equations (8) and (9), then squaring both sides and simplifying, we obtain for the equation of the curve:

$$y = h\,(1 - (x^2/b^2)) \tag{10}$$

The volume, V, of the droplet is

$$V = 2\,\pi \int_0^b y^2\,dx. \tag{11}$$

$$\begin{aligned} V &= 2\,\pi \int_0^b (h(1 - (x^2/b^2)))^2\,dx \\ &= 2\,\pi\,h^2 \int_0^b (1 - (x^2/b^2))^2\,dx \\ &= 2\,\pi\,h^2 \int_0^b (1 - 2\,(x/b)^2 + (x/b)^4)\,dx \\ &= 2\,\pi\,h^2\,(x - (2\,x^3/(3\,b^2)) + (x^5/(5\,b^4)))\Big]_0^b \end{aligned}$$

$$V = \frac{16\pi h^2 b}{15} \tag{4}$$

Substituting $b = l/2$ and $h = w/2$ to express the formula in terms of directly measured droplet dimensions, we obtain:

$$V = \frac{2\pi (w)^2 l}{15} \tag{1}$$

To check whether equation (2) or equation (4) provides the closest estimate of the volume of a parabola, we ran a numerical integration to simulate 1,000,000 parabola subsections added together using the program R (R Development

Table 1.—Mean dimensions and volumes of viscous thread droplets from five adult female *Argiope aurantia* at three humidities (± standard deviation).

Relative humidity (%)	Droplet length (µm)	Droplet width (µm)	Droplet width/length	Droplet volume (µm³)
17.8 ± 2.2	63.6 ± 5.5	45.0 ± 5.5	0.74 ± 0.04	54,950 ± 14,856
55.0 ± 0.7	67.2 ± 5.5	48.4 ± 5.3	0.71 ± 0.05	67,337 ± 19,002
90.8 ± 0.8	82.2 ± 7.5	61.0 ± 5.8	0.72 ± 0.05	130,381 ± 32,806

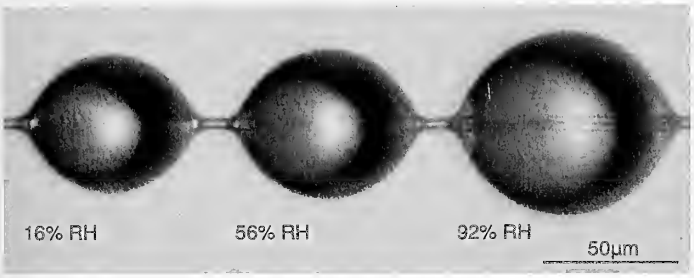

Figure 2.—Photograph of the same droplet of an individual *Argiope aurantia* at three humidities.

Core Team 2010). We used a quadrature rule based on interpolating functions with 1,000,000 permutations. Assigning an h value of 2 and b value of 5 we found an answer of ~ 67.020, which is what equation (4) finds, with equation (2) finding a much underestimated answer of ~ 8.378.

When we re-ran the simulation using an h value of 4 and b value of 10, we found an answer of ~536.135, which is consistent with what equation (4) finds. When $l = 2$ and $w = 5$, however, our answer of ~31.41 more closely approximates what equation (2) finds; confirming that there was an error in defining the variables h and b in formula (2).

APPLICATION

Does droplet shape change with humidity?—We examined the droplets from the viscous threads spun by five adult female *Argiope aurantia* Lucas 1833, whose threads were collected near Blacksburg, Montgomery Co., Virginia during September 2010. Using techniques described previously (Opell et al. 2011a), we transferred threads from web sectors to microscope slides and recorded the positions of individual droplets by identifying the position of a thread strand on the sampler and the position of the droplet by noting its sequential position from a support. We photographed each droplet at the same magnification at 17, 55, and 90% relative humidity, within a temperature range of 23–24 °C and measured droplet length and width with an OndeSoft® screen protractor (Beijing Torrentsoft Technology Co., Ltd. Beijing, China) using an image of a stage micrometer as a scale.

After inserting each of these 15 droplet images into a PowerPoint® (version 14.46 for Mac 2011, Microsoft Inc., Mountain View, CA) slide, we drew lines through the droplets' horizontal and vertical axes, and positioned 12 small points along the outline of the upper right quarter of the droplet such that they were equally spaced on the X axis that bisected the droplet (Fig. 1). We then recorded the coordinates of each point and used the two outermost points to construct a parabola. From each droplet's X coordinate, we computed its Y coordinate as predicted by the parabola and compared these predicted coordinates to the measured coordinates to determine if a droplet's shape matched that of a parabola. The outermost points on each droplet were excluded from the analysis, as these were used to compute the parabola contour against which the other ten points were compared.

At each humidity, droplet contour assumed the shape of a parabola (Fig. 2). The ratio of droplet width to length (Table 1, Fig. 3), which was normally distributed (Shapiro-Wilk test $P > 0.11$), did not differ among humidities (ANOVA $P = 0.5156$). Thus we confirmed that the formula described in this study is appropriate for across-humidity comparisons of droplet volumes.

CONCLUSION

The volume of a viscous thread droplet of an orb web can be reliably estimated as that of a parabola rotated around its axis by equation (4). Because the forces driving the formation of droplets are the same among orb web spiders, the formula can be universally applied, facilitating more meaningful comparisons between studies. It will be easier and less complicated, in practice, if the formula is expressed in terms of the droplet length and width dimensions l and w rather than the parabola coordinates h and b. By showing that a viscous droplet continues to assume the shape of a parabola over a wide range of humidities, we demonstrated that the formula has broad applicability for measuring the volumes of all spiral droplets. This test also shows how an investigator can confirm this fit in other situations.

ACKNOWLEDGMENTS

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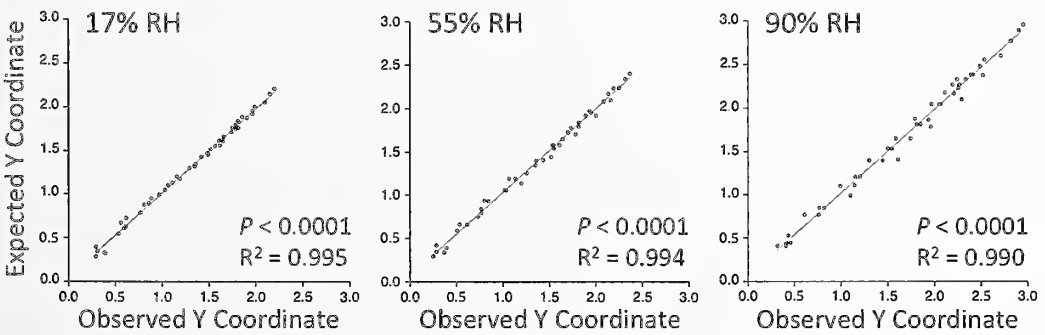

Figure 3.—Evaluation of the fit of *Argiope aurantia* viscous droplets to a parabola at three humidities. Each regression plots the expected and observed Y coordinates of ten points along the contour of each of the five individual's droplets.

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SHORT COMMUNICATION

Effects of non-native *Eucalyptus* plantations on epigeal spider communities in the northern Negev desert, Israel

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Abstract. Plantation forests are being planted at an increasing rate and account for 7% of the global forested area. The majority of planted forests are composed of exotic tree species, and *Eucalyptus* trees have become the most widely planted hardwood species in the world. While *Eucalyptus* plantations have economic importance, their role in native biodiversity conservation, especially in areas without naturally occurring forests, is little explored. In the present study, we assessed the impact on biodiversity of replacing natural semi-deserts with *Eucalyptus camaldulensis* plantations. The impact was evaluated by comparing epigeal spider communities of seven plantations with previously sampled communities of seven natural habitats in the northwestern Negev, Israel. In contrast to our assumptions, spider species richness was higher in *Eucalyptus* plantations compared to natural semi-deserts. However, substantial differences in species composition between the two habitat types were observed. Few species found in natural semi-deserts were sampled in the plantations, suggesting that *Eucalyptus* plantations cannot substitute for natural semi-desert habitats.

Keywords: Afforestation, spider, *Eucalyptus camaldulensis*, exotic plantation, land-use change

Today, the majority of planted forests are composed of exotic tree species, and *Eucalyptus* trees have become the most widely planted hardwood species (FAO 2006). However, the role of exotic *Eucalyptus* plantations in supporting native biodiversity outside Australia is controversial. Frequently, *Eucalyptus* plantations are considered as 'ecological deserts' (Broekerhoff et al. 2001), supporting fewer species than natural forests (e.g., Gardner et al. 2008) or natural open land habitats (Rodrigues et al. 2010; Gries et al. 2012).

Historically, the northern Negev desert in Israel is composed mainly of loess plains and steppe shrublands. Both semi-desert habitats are dominated by low and thick perennial shrubs, which are unique habitats for a variety of habitat specialists (Shochat et al. 2001). These natural habitats, however, have been mainly replaced by crop fields and more recently by exotic *Eucalyptus* plantations. Due to these anthropogenic influences, loess plains and steppe shrublands have become two of the rarest and most threatened habitats in Israel (SPNI 2014). While species richness and abundance of spiders has already been shown to be higher in natural semi-deserts than in crop fields (Pluess et al. 2008), little is known about the value of *Eucalyptus* plantations for spiders in this region. In the present study, we assessed the impact on species richness and abundance of spiders of replacing natural semi-deserts with *Eucalyptus camaldulensis* (Dehnh.) plantations in the northwestern Negev, Israel.

Spiders were sampled in seven *Eucalyptus camaldulensis* plantations. The sampled communities were then compared to spider communities sampled in seven natural semi-desert habitats by Pluess et al. (2008). The geographic locations of the *Eucalyptus* plantations sites were selected to vary as little as possible from the natural semi-deserts (Mann-Whitney-U test of latitudinal locations: $z = 0$, $p = 1$) as the latitudinal rainfall gradient has been shown to be correlated with species richness and abundance in plants, small mammals, insects and spiders (Opatovsky et al. 2010; Segev 2010). The sampling sites were distributed over an area of approximately 15 km × 10 km in the northwestern Negev, Israel (Fig. 1) within the Irano-Turanian biogeographic region (Segev 2010).

The *Eucalyptus camaldulensis* plantations were located along dry riverbeds and were planted by the Keren Kayemeth LeIsrael - Jewish National Fund (KKL-JNF) 12 to 55 years ago. *Eucalyptus* trees have been planted in densities of approximately one tree per 25–56 m^2 on areas varying between 1.1 ha and 5.2 ha. The ground was mainly covered with leaf litter, interspersed with bare ground and vegetation. If present, the ground vegetation consisted of grasses and herbaceous species. All plantations were unmanaged and adjacent to other forest plantations and crop fields. The natural semi-deserts were located along dry riverbeds or on borders of military training areas. The vegetation comprised scattered perennial shrubs and geophytes, grasses and herbaceous species that appeared after winter rains, and some sites were interspersed with recently planted trees. For more detailed information about the natural semi-desert sites see Pluess et al. (2008).

Pitfall traps were used to sample the *Eucalyptus* spider community in order to be compared with the pitfall-trap sampling of Pluess et al. (2008) in the semi-desert habitat. Sixteen pitfall traps per site were used in *Eucalyptus* plantations, and 20 pitfall traps per site in natural semi-deserts. The traps consisted of plastic cups, which were 10 cm deep with an opening diameter of 9 cm. The cups were buried in the ground in such a way that the rim was level with the ground surface. Each trap contained 150 ml of 50% ethylene glycol with a drop of detergent as trapping liquid. As in Pluess et al. (2008), the traps in *Eucalyptus* plantations were opened for one week in January and for one week in February. The sampling dates were selected according to the high spider activity in this region during the first months of the

This publication is dedicated to Keren Embar.

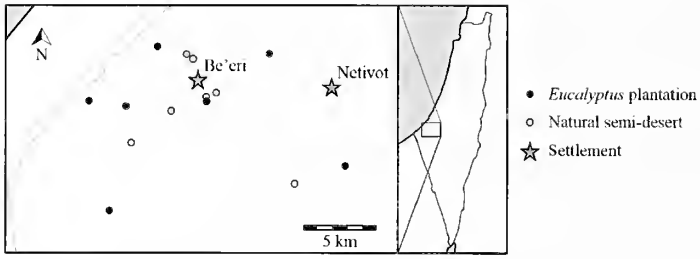

Figure 1.—Distribution of the 14 sampling sites in the northwestern Negev, Israel. Circles depict sampling sites.

year (Gavish-Regev et al. 2008). Spider communities in *Eucalyptus* plantations were sampled in 2011 and compared to the spider communities sampled in natural semi-deserts in 2007 (Pluess et al. 2008). For each site, the captures of both sampling sessions and all traps were pooled prior to analyses. We identified all individuals to family level and adult individuals to species or morphospecies level. The nomenclature followed Platnick (2013). Voucher specimens are deposited in the Arachnid Collection at the Mitrani Department of Desert Ecology, Ben-Gurion University of the Negev and in the National Arachnid Collection at the Hebrew University of Jerusalem, Israel. Only adult individuals were used for the statistical analyses.

Species-accumulation curves were used to compare species richness among habitats using rarefaction (Gotelli & Colwell 2001). Because pitfall traps were pooled prior to identification, an individual-based rather than sample-based approach was used for rarefaction. The implemented algorithm was based on a log Gamma function (Krebs 1989). The estimated mean and standard errors were used to estimate 95% confidence intervals. A significant difference in the total observed species richness of one habitat type was inferred if it fell outside of the 95% confidence interval of the other habitat type. An average of first-order Jackknife (Jack1) (Burnham & Overton 1978), first-order Chao (Chao1) (Chao 1987) and ACE (Abundance-based Coverage Estimator; Collwell & Coddington 1994) were used to estimate true species richness for each habitat. The species coverage of each habitat was assessed by calculating the number of observed species as a percentage of this estimate (Lobo 2008). Analyses of similarities (ANOSIM) were performed on the basis of Horn-Morisita similarities to test for significant differences of spider species composition between the two habitat types (R = 0 indicates complete similarity, R = 1 indicates complete dissimilarity). Nine morphospecies (singletons and doubletons) were omitted from ANOSIM because the taxonomic identity of individuals sampled in the two habitat types was unclear (see species with '?' in the occupancy columns in Appendix 1). Horn-Morisita similarities of transformed data were used to account for different sample sizes (Chao et al. 2006).

Rarefaction curves and the Mann-Whitney U test were performed using PAST (Hammer et al. 2001). The remaining analyses were performed using R (R Development Core Team 2012). We used the "fossil" package (Vavrek 2010) to calculate all richness estimators and the "vegan" package (Oksanen et al. 2010) to calculate ANOSIM.

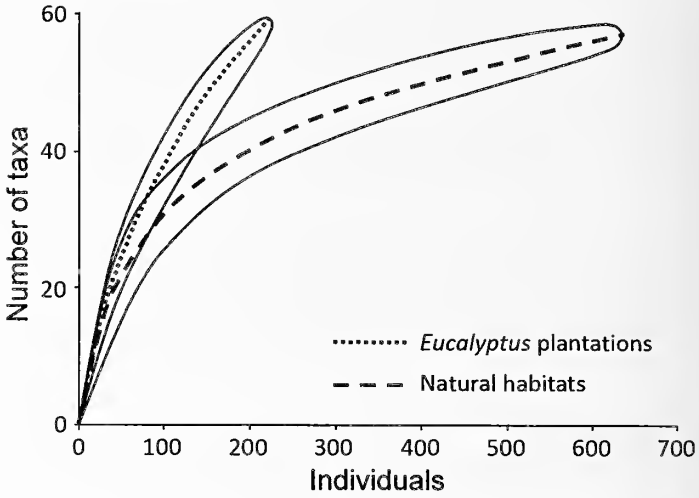

Figure 2.—Individual-based rarefaction curves for spiders in *Eucalyptus* plantations and natural semi-desert. Solid lines indicate 95% confidence intervals.

A total of 327 spiders were sampled during 1407 trapping days in *Eucalyptus* plantations. The 210 adult individuals belonged to 59 species in 21 families. In comparison, Pluess et al. (2008) sampled 1008 spiders during 1820 sampling days in surrounding natural semi-deserts. The 642 adult individuals belonged to 58 species in 16 families (Appendix 1). Rarefaction curves and coverage suggest significantly higher species richness in *Eucalyptus* plantations than in natural semi-deserts (Fig. 2, Table 1). In contrast, activity density was more than twice as high in semi-deserts compared to *Eucalyptus* plantations (Table 1).

The ANOSIM revealed significant differences of spider communities in *Eucalyptus* plantations and natural semi-deserts ($R = 0.573$, $P < 0.001$). In *Eucalyptus* plantations, 61.8% of the species were exclusive to this habitat and 62.9% of the species sampled in natural semi-deserts were not found in *Eucalyptus* plantations (Appendix 1). Only 20 spider species were found in both habitats. Three families Idiopidae, Oonopidae, and Sicariidae, were sampled only in *Eucalyptus* plantations (Appendix 1). In contrast, individuals of the family Zoridae were sampled only in natural semi-deserts.

The comparably high species richness in *Eucalyptus* plantations contradicts results of earlier studies, which observed lower species richness of Araneae and Scarabeidae in *Eucalyptus* plantations compared to natural open-land habitats (Rodrigues et al. 2010; Gries et al. 2012). Further, spider communities in *Eucalyptus* plantations differed strongly from those in natural semi-deserts. Fewer than half of the species were common to both habitats (Appendix 1) and the magnitude of calculated dissimilarities to natural semi-deserts is comparable to dissimilarities between natural forests and open-land (Kajak & Łukasiewiez 1994). This is in clear contrast to other results showing that *Eucalyptus* plantations in non-native regions mainly contain subsets of species sampled in natural habitats (Gardner et al. 2008).

Table 1.—Sampling effort, activity density, and species richness for spiders sampled in *Eucalyptus* plantations and natural semi-deserts. Column superscripts: [a] number of sampling days multiplied by number of intact traps; [b] mean number of juvenile and adult spiders per trap (± SE); [c] number of species observed; [d] number of species rarefied for 180 individuals (± SE); [e] number of species observed as percentage of estimated species richness (average Chao 1, Jack 1, and ACE).

Habitat type	Trap days[a]	Activity density[b]	S_{obs}[c]	S_{rar180}[d]	Coverage[e]
Eucalyptus plantations	1407	1.6 (± 0.1)	59	57.3 (± 0.9)	62.7
Natural semi-deserts	1820	3.8 (± 0.6)	58	39.7 (± 2.6)	80.2

These unexpected results may be explained by the exceptional role of *Eucalyptus* plantations in southern Israel. In the northern Negev, natural forests were absent for a long period of ecological time (Ginsberg 2002) and the majority of *Eucalyptus* trees are planted along dry riverbeds, increasingly replacing remaining natural semi-desert habitat (Amir & Rechtman 2006; SPNI 2014). This change in landscape structure can influence the local biodiversity in two ways: On the one hand, the afforestation with *Eucalyptus* trees transformed the once continuous natural semi-deserts into isolated habitat patches (Amir & Rechtman 2006). This increasing isolation of natural habitat may enhance the negative effect of habitat loss on remaining spider populations (Herrmann et al. 2010, 2012) by breaking continuous populations into metapopulations (Hanski & Gilpin 1991) or source-sink populations (Pulliam 1988) and increasing the negative effects of stochastic processes (reviewed by Simberloff 1994). Isolation effects may have led to a loss of species in natural semi-deserts, resulting in impoverished spider communities. On the other hand, the afforestation along dry riverbeds creates a well-connected web of plantations. This connectivity of plantations facilitates species dispersal (Calçada et al. 2013) and increases species richness in connected habitat patches (Bailey et al. 2010). The plantations, however, create "institutionalized landscapes", different and foreign to the local vegetation (Amir & Rechtman 2006). They offer new structures and microenvironments, which are known to favor species that are not found in open land habitats (Uetz 1979). Instead of supporting species occurring in natural semi-deserts, *Eucalyptus* plantations may expand the natural distribution of forest species occurring in the central and northern part of Israel. Similar patterns have been observed in other parts of the Negev, where Mediterranean bird species immigrated from central and northern parts of Israel to establish populations in exotic coniferous plantations (Shochat et al. 2001).

Despite the relatively high species richness, the spider abundance in *Eucalyptus* plantations was comparatively low (Table 1). The low activity density could be linked to the biology of *Eucalyptus* trees. *Eucalyptus*-produced biomass is mostly unpalatable to native organisms in regions where these trees have been introduced (Paine et al. 2011). As herbivorous and detritivorous fauna are major food sources for epigeal spiders (Foelix 1996), large amounts of biomass and energy produced by *Eucalyptus* are hardly transferred to higher trophic levels (Cordero 2011).

Even though *Eucalyptus* plantations and natural semi-deserts were sampled at the same time of the year, *Eucalyptus* plantations were sampled in a different year than the natural semi-deserts. Differences in climate between the years may have influenced the phenology of some spider species (Polis & Yamashita 1991), thereby biasing our habitat comparison. In arid ecosystems, rainfall is most likely to cause differences by stimulating plant growth, animal activity and reproduction (James et al. 1995; Langlands et al. 2006). In the present study, total rainfall during the sampling and three months prior to the sampling was much lower in the year of *Eucalyptus* plantation sampling compared to the year of natural semi-desert sampling (5 month total: *Eucalyptus* sampling: 149 mm; semi-desert sampling: 249 mm). Recent studies in arid ecosystems showed an increase of spider abundance in years with higher precipitation (Langlands et al. 2006). The low precipitation during the sampling of *Eucalyptus* plantations may therefore have contributed to the low spider abundance. Yet, no significant relationship between precipitation and spider species richness has been found (Langlands et al. 2006; Opatovsky et al. 2010). Hence, the lower precipitation is unlikely to explain the higher species richness in *Eucalyptus* plantations.

Despite the frequently cited assumption of being 'ecological deserts' (Brockerhoff et al. 2001), our results indicate a higher spider species richness in *Eucalyptus* plantations compared to natural semi-deserts. However, since spider community dissimilarities were high between the two habitats and only few semi-desert species actually inhabited plantations, *Eucalyptus* plantations cannot substitute for natural semi-deserts. The continuing replacement of natural semi-deserts with *Eucalyptus* plantations may therefore lead to fundamental changes of spider communities in this region.

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Appendix 1.—List of taxa sampled in *Eucalyptus* plantations and natural semi-deserts. Values represent number of sites each taxon was sampled from. '?'s indicate unclear taxanomic identy within a family between the two habitats (e.g., within Dictynidae, Morphospecies 1 is either the same as or different from Morphospecies 2).

Family	Taxon	Occupancy in	
		Eucalyptus	Natural habitat
Araneidae	Morphospecies 1	1	0
Clubionidae	Morphospecies 1	1	0
	Morphospecies 2	1	0
	Clubiona genevesis (L. Koch, 1866)	0	2
Corinnidae	Morphospecies 1	0	1
Ctenidae	*Anahita* sp.	2	0
Dictynidae	Morphospecies 1	1	?
	Morphospecies 2	?	2
Dysderidae	Morphospecies 1	1	0
	Dysdera sp.	0	2
	Dysdera westringi (O. P.-Cambridge, 1872)	1	6
	Harpactea sp.	1	4
	Tedia abdominalis (Deeleman-Reinhold, 1988)	2	2
	Tedia oxygnatha (Simon, 1882)	0	1
Filistatidae	Morphospecies 1	2	4
Gnaphosidae	Morphospecies 1	1	0
	Morphospecies 2	1	0
	Morphospecies 3	1	0
	Haplodrassus mediterraneus (Levy, 2004)	0	1
	Haplodrassus morosus (O. P.-Cambridge, 1872)	2	2
	Micaria corvina (Simon, 1878)	4	4
	Micaria ignea (O. P.-Cambridge, 1872)	0	1
	Micaria pallipes (Lucas, 1846)	0	1
	Minosia spinosissima (Simon, 1878)	6	1
	Odontodrassus nunudus (O. P.-Cambridge, 1872)	0	2
	Talanites sp.	0	1
	Zelotes laetus (O. P.-Cambridge, 1872)	1	0
Idiopidae	*Idiops syriacus* (O. P.-Cambridge, 1870)	2	0
Linyphiidae	Morphospecies 1	1	?
	Morphospecies 2	1	?
	Morphospecies 3	1	?
	Morphospecies 4	1	?
	Morphospecies 5	?	2
	Erigoninae 1	0	3
	Erigoninae 2	0	4
	Linyphiinae 1	?	1
	Linyphiinae 2	?	1
	Alioranus pastoralis (O. P.-Cambridge, 1872)	1	7
	Dicymbium sp.	3	0
	Meioneta pseudorurestris (Wunderlich, 1980)	0	4
	Pelecopsis sp. 1	0	3
	Pelecopsis sp. 2	0	1
	Pelecopsis inedita (O. P.-Cambridge, 1875)	1	0
Liocranidae	Morphospecies 1	0	1
	Liocranum sp. 1	2	3
	Mesiotelus sp. 1	1	2
Lycosidae	Morphospecies 1	2	7
	Morphospecies 2	2	0
	Alopecosa albofasciata (Brullé, 1832)	0	4
	Hogna sp.	2	0
	Pardosa proxima (C. L. Koch, 1847)	0	1
	Trochosa sp.	0	1
	Xerolycosa sp. 1	5	0
	Xerolycosa sp. 2	1	0
Oonopidae	*Opopaea* sp. 1	2	0
	Orchestina sp. 1	1	0
Philodromidae	*Thanatus meronensis* (Levy, 1977)	1	0
	Thanatus sp. 1	0	1
	Thanatus vulgaris (Simon, 1870)	1	2

Appendix 1.—Continued.

Family	Taxon	Occupancy in *Eucalyptus*	Occupancy in Natural habitat
Salticidae	*Aelurillus aeruginosus* (Simon, 1871)	0	2
	Aelurillus gershomi (Prószyn'ski, 2000)	2	0
	Aelurillus kochi (Roewer, 1951)	3	0
	Aelurillus politiventris (O. P.-Cambridge, 1872)	0	1
	Chalcoscirtus infimus (Simon, 1868)	1	0
	Pellenes sp.	0	1
	Pellenes geniculatus (Simon, 1868)	1	1
	Salticus propinquus (Lucas, 1846)	1	4
	Thyene sp.	1	0
Scytodidae	*Scytodes* sp.	4	0
Sicariidae	*Loxosceles rufescens* (Dufour, 1820)	1	0
Sparassidae	*Micromata formosa* (Pavesi, 1878)	2	1
Theridiidae	Morphospecies 1	1	0
	Morphospecies 2	1	0
	Enoplognatha sp.	0	1
	Enoplognatha genuina (Bosmans & Van Keer, 1999)	5	6
	Enoplognatha macrochelis (Levy & Amitai, 1981)	0	5
	Euryopis episinoides (Walckenaer, 1847)	0	1
	Steatoda albomaculata (De Geer, 1778)	1	0
	Steatoda paykulliana (Walckenaer, 1805)	0	3
	Platnickina nigropunctata (Lucas, 1846)	0	1
Thomisidae	*Ozyptila omega* (Levy, 1975)	0	2
	Ozyptila patellibidensis (Levy, 1999)	2	4
	Ozyptila rigida (O. P.-Cambridge, 1872)	1	0
	Ozyptila sp. 1	0	1
	Ozyptila sp. 2	0	4
	Ozyptila tricoloripes (Strand, 1913)	3	2
	Xysticus bliteus (Simon, 1875)	0	2
	Xysticus cristatus (Clerck, 1757)	1	3
	Xysticus edax (O. P.-Cambridge, 1872)	0	1
	Xysticus xerodermus (Strand, 1913)	2	3
Zodariidae	*Lachesana rufiventris* (Simon, 1873)	4	0
	Ranops expers (O. P.-Cambridge, 1876)	0	4
	Trygetus sexoculatus (O. P.-Cambridge, 1872)	3	0
	Zodarion nitidum (Audouin, 1826)	1	2
Zoridae	*Zoropsis lutea* (Thorell, 1875)	0	1
Unknown	Morphospecies 1	1	0
	Morphospecies 2	2	0

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SHORT COMMUNICATION

Predator cues have contrasting effects on lifespan of *Pardosa milvina* (Araneae: Lycosidae)

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Abstract. Predators can affect prey indirectly by eliciting changes in behavior, morphology, and life history. These nonconsumptive effects are often mediated by predator cues used by prey to avoid capture. However, predator cues can cause stress responses in prey that negatively impact survival and reproduction. We explored responses of the wolf spider *Pardosa milvina* (Hentz 1844) to cues from the larger wolf spider *Tigrosa helluo* (Walckenaer 1837) and the ground beetle *Scarites quadriceps* Chaudoir 1843. We exposed *Pardosa* to cues from both predators and measured changes in body size, weight, consumption, and lifespan. We found significant effects of predator cues only on female longevity: females exposed to *Tigrosa* cues had shorter lifespans than those exposed to cues from *Scarites*. The lack of treatment effects on energy intake suggests that predator cues act through physiologieal pathways. Future experiments may uncover opposing hormonal mechanisms underlying the observed differences in lifespan.

Keywords: Nonconsumptive effects, foraging, predation, *Tigrosa helluo*, *Scarites quadriceps*

Predation is a ubiquitous ecological interaction that shapes the behavior, morphology, physiology, and life history of both predators and prey. Although traditionally focused on the consumption of prey by predators (i.e., consumptive effects), researchers have gained an appreciation for the impacts predators can have on prey without consuming them (i.e., nonconsumptive effects; Werner & Peacor 2003; Preisser et al. 2005). These nonconsumptive effeets are frequently mediated by cues deposited by predators, often created as a by-product of interactions with prey (e.g., kairomones; Dicke & Grostal 2001). Prey have evolved the ability to respond to predator cues by altering their behavior and morphological traits (Benard 2004), but these modifications come with associated costs. Reduced foraging is a common trade-off made by prey responding to the risk of predation, and often translates into reduced growth, development, and survival (Lima 1998; Relyea 2007; Hawlena & Schmitz 2010a), though compensatory feeding is possible (Thaler et al. 2012; Hawlena & Schmitz 2010b).

Interestingly, stress induced by predator cues can deerease prey development and growth and increase prey mortality independent of changes in foraging (Stoks 2001; McCauley et al. 2011; Siepielski et al. 2014). If predator presence leads to a decrease in prey population sizes without directly consuming or changing the foraging success of their prey, then our conceptual models of predator-prey dynamics may be incomplete due to incorreetly attributing prey mortality to consumptive effects (Peckarsky et al. 2008; McCauley et al. 2011). Understanding the nonconsumptive effects predators have on their prey is an important goal in ecology, as prey responses to the risk of predation can affect processes at scales exceeding prey physiology and behavior by altering food web structure and ecosystem function (Hawlena & Schmitz 2010a). To further research on noneonsumptive predator effects, we explored the impacts of cues from two predators on the foraging, development, and survival of their shared prey using a well-studied system.

The wolf spider (Araneae: Lycosidae) *Pardosa milvina* (Hentz 1844) co-occurs with two larger predators: the wolf spider *Tigrosa helluo* (Walckenaer 1837), formerly *Hogna helluo* (Walckenaer 1837) (see Brady 2012), and the ground beetle (Coleoptera: Carabidae) *Scarites quadriceps* Chaudoir 1843 (study species hereafter referred to by genus). *Pardosa* has evolved a sophisticated sensory system able to detect nuanced information in cues deposited by *Tigrosa* indicating predator size (Persons & Rypstra 2001), sex (Lehmann et al. 2004), hunger level (Bell et al. 2006), diet (Persons et al. 2001), and residency status (Barnes et al. 2002). Although not as well-characterized, *Pardosa* does respond to cues from *Scarites* with decreased patch residence time (Wrinn et al. 2012) and moderate inereases in activity (Sitvarin, unpublished data). In contrast, *Pardosa* respond to *Tigrosa* cues with increased patch residence time (Wrinn et al. 2012) and decreases in activity (Persons et al. 2002; Sitvarin, unpublished data). Therefore, evidence suggests these predators are functionally inverse (i.e., cause opposing responses in prey, Herzog & Laforsch 2013), and thus are likely to cause conflicting responses in *Pardosa* foraging, development, and survival.

We expected exposure to predator cues to alter prey consumption, development, and survival of *Pardosa*. Specifically, we predicted that cues from *Tigrosa* would elicit responses opposite those seen in spiders exposed to *Scarites* cues, with *Tigrosa* cues resulting in increased prey consumption due to lower body condition (Lima & Bednekoff 1999; Persons et al. 2002), slower development, and decreased lifespan due to documented negative effects of *Tigrosa* cues on *Pardosa* (Persons et al. 2002; Taylor et al. 2005; Folz et al. 2006; Rypstra et al. 2007). Furthermore, we expected that longer exposure to predator cues would strengthen responses as chronic predator stress has larger effects than acute exposure (Lima 1998; Hawlena & Schmitz 2010a). Finally, we predicted males to be more strongly affected by the treatments due to previously documented differences between sexes in responses to experienee with predator cues (Sitvarin & Rypstra 2012).

We eollected all study organisms from Miami University's Ecology Research Center (39° 31′ 33″ N, 84° 43′ 20″ W). Female *Pardosa* carrying eggsacs were maintained individually in translucent containers (5.5 cm high × 5.5 cm diameter) with a 2 cm deep layer of moistened soil/peat moss mixture. Adult female *Tigrosa* and adult *Scarites* were maintained individually in larger containers (8 cm high × 12 cm diameter) with the same substrate type. Water was available *ad libitum*, and two crickets (*Acheta domesticus* Linnaeus, 1758), approximately half the size of the predator, were provided weekly. All containers were maintained in an environmental chamber on a 13L:11D light cycle at 25°C.

When *Pardosa* spiderlings dispersed (approximately two weeks after eggsacs hatched), we took no more than 12 spiderlings from each clutch (n = 21 clutches) and placed them individually into separate cultures containing collembolans (*Sinella curviseta*) as prey. After three molts, we transferred spiderlings to new containers and fed them two appropriately-sized crickets weekly. *Pardosa* reached the penul-

Table 1.—The effects of cue source (none, *Tigrosa helluo*, or *Scarites quadriceps*), exposure duration (1 or 3 days), and their interaction on female and male *Pardosa milvina* lifespan.

	Females			Males		
	df	*F*	*P*	df	*F*	*P*
Cue source	2	4.02	0.030	2	1.60	0.224
Duration	1	0.17	0.683	1	0.33	0.574
Cue source*Duration	2	3.13	0.060	2	1.42	0.264

timate stage after 70.8 ± 2.9 days (mean ± SE) and were randomly assigned to one of six treatments in a factorial design: one of three predator cue exposures (none, *Tigrosa* cues, or *Scarites* cues) and one of two exposure durations (1 or 3 days). We used penultimate spiders because the transition to adulthood may represent an especially sensitive period in life history (McCauley et al. 2011).

Three weeks prior to experimentation, we provided each *Tigrosa* and *Scarites* three crickets and then deprived them of food until trials were completed. We exposed *Pardosa* to predator cues by removing *Tigrosa* and *Scarites* from their containers and placing a single *Pardosa* into each container, thus preventing predation while allowing *Pardosa* to sense predator cues (i.e., silk, feces, and other excreta). To evaluate potential stress effects on body condition, we measured the abdomen width and weight of each *Pardosa* before and immediately after the exposure period 1 d or 3 d). Additionally, we provided each spider with two crickets after exposure and recorded change in abdomen width and weight after two days. We returned *Pardosa* to their original containers after the exposure period, recorded the number of days required to reach the final molt to adulthood, and monitored survival three times weekly until death.

Due to differences in longevity (Foelix 1996) and previously documented behavioral and developmental differences between males and females (Sitvarin & Rypstra 2012), we analyzed each sex separately. We evaluated the effects of predator cue source, exposure duration, and their interaction on *Pardosa* lifespan using linear mixed-effects models. We used cue source and exposure duration as fixed effects and clutch as a random effect to predict post-exposure lifespan, where a significant interaction between cue source and exposure duration indicates that the effect of predator cues depends on how long *Pardosa* is exposed. Differences in longevity between treatments were tested using one-way ANOVA followed by pairwise Tukey HSD comparisons. Changes in abdomen width and weight due to cue exposure and post-exposure feeding were tested separately with two-way ANOVA, using predator cue source and exposure duration as factors. The number of days required until the final molt to adulthood was similarly analyzed with two-way ANOVA. All analyses were conducted using R (R Core Team 2013).

Female *Pardosa* post-exposure longevity (144.9 ± 8.8 days) was significantly affected by predator cue source and marginally impacted by the interactive effects of predator cue source and exposure duration (Table 1). Specifically, females had shorter lives after encountering *Tigrosa* cues for three days compared to those exposed to *Scarites* cues for three days, though neither treatment was significantly different from the blank treatments (Fig. 1a). Exposure to cues from these predators for one day had no effect on female longevity, and spiders from those treatments had eomparable lifespans to those in the blank treatments. In contrast to the effects on female spiders, male post-exposure lifespan (87.3 ± 4.7 days) was unaffected by any experimental treatment (Table 1, Fig. 1b).

There was no effect of predator cue source or exposure duration on changes in abdomen width or weight after exposure or after feeding on crickets (females: all $P > 0.4$, males: all $P > 0.2$) or the number of days required for spiders to reach adulthood (females: $P > 0.5$, males: $P > 0.7$) (Table 2).

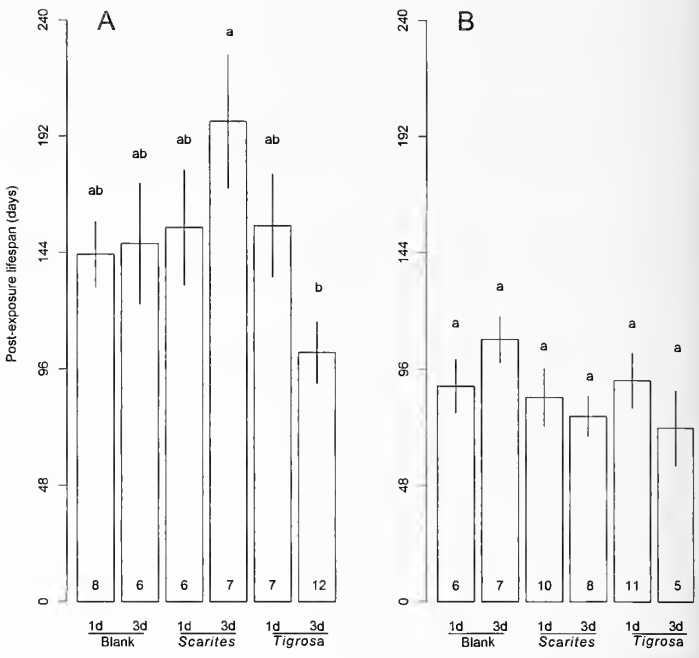

Figure 1.—The effects of exposure to cues from *Tigrosa helluo*, *Scarites quadriceps*, or no cues for one or three days on the mean (±SE) post-exposure lifespan of *Pardosa milvina* females (A) and males (B). Treatments sharing the same letter do not differ significantly. Sample sizes appear in bars.

We have demonstrated that exposure to cues from predators can have lifespan-altering effects on prey. Interestingly, the two predators used induced opposite responses that were sex-specific. Female *Pardosa* exposed to *Tigrosa* cues had significantly shorter lifespans than those exposed to cues from *Scarites*, though male spiders were unaffected by predator cues. To our knowledge, a predator cue-based increase in longevity has never been demonstrated before, though reduced lifespans after exposure to predator cues are known (e.g., McCauley et al. 2011).

We found reductions in female lifespan despite a lack of impact on body size, weight, or development. Similarly, larval odonates exposed to predator cues have been found to have increased mortality rates compared to unexposed individuals (Stoks 2001; McCauley et al. 2011; Siepielski et al. 2014). In one study, reduced damselfly lifespan was accompanied by slower growth and development rate, and smaller, more asymmetric wings after metamorphosis (Stoks 2001). However, these detrimental effects on prey beyond decreased longevity are not always present: prey exposed to predator cues had lower survival but did not differ from the control treatment in terms of body size as larvae or adults (McCauley et al. 2011; Siepielski et al. 2014). Interestingly, we did not find evidence for reduced foraging to explain how predator cues may reduce prey lifespan; odonates were also found to have unaltered energy intake in the presence of predator cues (Stoks 2001; McCauley et al. 2011; Siepielski et al. 2014). Prey typically must make trade-offs between anti-predator behavior and foraging, so most responses (e.g., reduced body size, slower development) are attributed to decreased energy intake (Lima 1998; Persons et al. 2002; Benard 2004; Relyea 2007; Hawlena & Schmitz 2010b; but see Davenport et al. 2014). However, in our study and the odonate studies described above, prey had *ad libitum* access to food (Stoks 2001; McCauley et al. 2011; Siepielski et al. 2014) or were provided a standardized amount of food without the presence of competitors, thus making reduced energy intake an unlikely explanation for decreased longevity. Although increased survival has been observed in response to predator cues, this effect is due to thinning (*sensu* Relyea 2007), a phenomenon unrelated to our results due to the lack of interactions among spiders.

Table 2.—Mean (±SE) change (post – pre) in abdomen width (abd., mm) and weight (wgt., μg) after exposure (exp.) to cues from *Tigrosa helluo*, *Scarites quadriceps*, or no cues for one or three days and after foraging (feed) and number of days to reach adulthood for female and male *Pardosa milvina*.

	Females					Males				
	Abd. exp.	Wgt. exp.	Abd. feed	Wgt. feed	Days	Abd. exp.	Wgt. exp.	Abd. feed	Wgt. feed	Days
Blank 1d	−0.07	−8.4	0.08	58.8	226.7	−0.16	−7.2	0.16	6.6	173.8
	(0.06)	(5.4)	(0.03)	(4.6)	(13.4)	(0.06)	(1.7)	(0.03)	(2.9)	(14.2)
Blank 3d	−0.14	−9.7	0.10	103.3	225.2	−0.02	−4.3	−0.23	−6.5	191.0
	(0.05)	(1.7)	(0.02)	(2.8)	(24.6)	(0.05)	(2.5)	(0.27)	(14.2)	(10.7)
Tigrosa 1d	−0.11	−15.4	0.09	85.7	241.1	−0.03	−4.6	0.09	4.6	174.6
	(0.06)	(4.1)	(0.04)	(2.3)	(21.4)	(0.07)	(5.3)	(0.04)	(2.7)	(12.3)
Tigrosa 3d	−0.08	−5.0	0.09	40.0	187.0	−0.08	−7.6	0.03	6.0	160.2
	(0.03)	(3.7)	(0.03)	(3.7)	(12.4)	(0.06)	(2.7)	(0.05)	(2.5)	(17.9)
Scarites 1d	−0.11	−2.0	0.13	122.5	236.5	−0.11	−1.9	0.12	6.1	169.5
	(0.14)	(2.8)	(0.07)	(4.2)	(24.5)	(0.09)	(2.1)	(0.03)	(2.1)	(10.4)
Scarites 3d	−0.18	−11.7	0.02	116.7	289.0	−0.12	−0.7	0.03	−2.0	153.8
	(0.09)	(6.3)	(0.09)	(3.2)	(28.0)	(0.05)	(11.2)	(0.07)	(11.3)	(9.5)

The precise proximate mechanism underlying changes in longevity after exposure to predator cues is unknown. Changes in foraging are unlikely (see above), and we saw no evidence of mortality attributable to pathogens (e.g., fungi or nematodes), both of which are mechanisms previously implicated as explanations for shortened lifespan after exposure to predator cues (McCauley et al. 2011). Stress hormones are likely involved, as the stress response diverts resources from other processes such as body maintenance, growth, and reproduction (Hawlena & Schmitz 2010a). We detected no effects on growth or development, so changes in behavior (e.g., Persons et al. 2002) or hormone-driven metabolic processes are likely mechanisms underlying the observed responses. Spiders exposed to predator cues may have altered assimilation efficiencies (Thaler et al. 2012), thus allowing differences in physiology that may translate into differential survival despite similar rates of growth and development.

Hormones coordinate large suites of behavioral processes and are known to regulate aetivity patterns in spiders. Specifically, the neurohormones serotonin and octopamine have contrasting effects on the huddle response of the orb-weaver *Larinioides cornutus* (Clerck 1757) (Araneae: Araneidae), lengthening and shortening the anti-predator behavior, respectively (Jones et al. 2011). Thus, there is a possibility that the contrasting effects of *Tigrosa* and *Scarites* cues on female longevity are driven by hormones acting in opposition. Discovering the hormonal underpinnings of this response could also provide insight on previously described opposing responses to cues from these predators in both emigration (Wrinn et al. 2012) and overall activity (Sitvarin, unpublished data). Increased longevity in response to predator cues has not previously been documented, but may be related to interactions between *Tigrosa* and *Scarites*. *Tigrosa* is the more dangerous predator for *Pardosa* (Sitvarin, unpublished data), though *Scarites* is capable of interfering with and consuming *Tigrosa* (Sitvarin & Rypstra 2014). Therefore, *Pardosa* may interpret *Scarites* cues as a forthcoming reduction in predation risk, leading to decreased levels of stress hormones or elevated levels of hormones that counter the effects of stress hormones.

We only found impacts of predator cues on lifespan for female spiders, a phenomenon likely tied to differences in life history between the sexes. Our sample sizes were modest, so the lack of effect on males may be a statistical artifact. However, males had significantly shorter lives than females, and so may also have less plasticity in lifespan. The sexes differ fundamentally in ecology (Foelix 1996) and in their growth and development trajectories (Sitvarin & Rypstra 2012). Furthermore, males have a higher metabolic rate than females (Walker & Irwin 2006), a fact that may put a limit on changes in longevity due to predator cues. Despite the lack of effect on males, they do exhibit a greater behavioral response to predator cues (Schonewolf et al. 2006; Sitvarin & Rypstra 2012) than females.

Further work is necessary to fully elucidate the interactions among these species. It would be worthwhile to characterize the way *Pardosa* responds to the simultaneous presentation of cues from *Tigrosa* and *Scarites*, as both activity and anti-predator behavior can change when cues from both predators are present (Sitvarin, unpublished data). There may also be interesting interactions with other stressors, such as food stress or autotomy (Stoks 2001), that provide insight into how prey cope with multiple demands in nature. Cues from *Tigrosa* are known to reduce courtship (Taylor et al. 2005) and foraging (Rypstra et al. 2007) in *Pardosa*, but the impact of *Scarites* cues remain largely unexplored. Furthermore, cues from these predators may have opposing effects on reproductive success of *Pardosa*, with the potential for differential effects on males and females. We still have much to learn about predation risk-induced stress hormones in invertebrates (Preisser 2009), which is particularly profound considering the potential for nonconsumptive interactions to drive evolution (Siepielski et al. 2014).

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SHORT COMMUNICATION

Vibration as an effective stimulus for aversive conditioning in jumping spiders

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Abstract. Previous work has shown that jumping spiders are able to associate visual cues with shock. We tested the efficacy of vibration as an aversive stimulus. *Phidippus audax* (Hentz 1845) (Salticidae) were first allowed to choose between two video stimuli, a cricket and an oval. We then tethered spiders so they were oriented toward their preferred stimulus with their tarsi touching a platform, either vibrated by a motor (experimental group) or with the motor turned off (control group). Spiders were then given a second opportunity to choose between the stimuli. Experimental spiders were significantly less likely to choose the stimulus that they viewed during training compared to control spiders. Spiders stalked and ate prey soon after experiencing the training procedure, suggesting that vibration caused no lasting harm. In addition, freely moving spiders avoided a vibrating platform, supporting the assertion that the vibration itself is aversive.

Keywords: Associative learning, training, Salticidae

Aversive conditioning can be an effective protocol for investigating animal cognition. In aversive conditioning, a negative stimulus (also called an unconditioned stimulus), such as shock, is paired with a neutral stimulus such as an image or tone. After training, animals avoid the previously neutral stimulus (now called the conditioned stimulus). While vibration has been used in studies of invertebrate learning, it is more often used as a conditioned stimulus rather than an unconditioned stimulus. For example, antlion larvae (*Myrmeleon crudelis*) learned to associate a vibrational cue with the arrival of food (Guillette et al. 2009), earthworms (*Lumbricus terrestris*) learned that substrate vibration predicted the onset of a bright light (Ratner & Miller 1959; Watanabe et al. 2005), and honeybees (*Apis melifera*) learned that vibration predicted electric shock (Abramson 1986). Here we test vibration as an aversive, unconditioned stimulus for studies of spider learning.

We studied vibration for four reasons. First, animals often learn more efficiently about stimuli that have some biological relevance (Shettleworth 2010). Spiders sense air- and substrate-borne vibration using, respectively, trichobothria and slit sensilla in the tarsal cuticle (Foelix 2011). Spiders use vibration in mating displays (reviewed in Sivalinghem et al. 2010) and to detect the presence of predators and prey (reviewed in Foelix 2011). Second, vibration has been used effectively as an unconditioned stimulus in several studies of spider learning. In *Araneus diadematus* Clerck 1757 spiders learned to associate different frequencies with aversive or non-aversive prey (Bays 1962). Spiders also attend to vibration as part of a multimodal cue: vibration enhanced the ability of jumping spiders, *Habronattus dossenus* Griswold 1987, to learn a color discrimination task (VanderSal & Hebets 2007). Third, vibration can be consistently administered across animals and trials because it is easy for the experimenter to see when the spider is experiencing the stimulus. Finally, vibration chambers are inexpensive and easy to build. The apparatus described here required minimal assembly and cost under $40 USD.

We collected adult and penultimate *Phidippus audax* (Hentz 1845) from fields and structures in Hampshire County, Massachusetts, USA, during late summer and early fall of 2013 and spring of 2014. Spiders were housed in 18 × 13 × 10 cm high clear plastic cages with a green wooden dowel, a refuge tube and plastic foliage for enrichment (Carducci & Jakob 2000). Spiders were fed crickets (*Acheta domesticus*) weekly and had constant access to water in cotton-stoppered test tubes. Spiders were starved for no less than four days before training and choice tests.

Our experimental design was to run initial choice tests to ascertain which of a pair of stimuli a naïve spider approached when given a choice, train the spider to associate vibration with its chosen stimulus, and finally give it a post-training choice test between the same two stimuli. The stimuli used were a silhouette of a cricket and a solid black oval (Fig. 1), which we knew from other work that *P. audax* could differentiate. We created stimuli in Adobe Illustrator CC and used ImageJ to adjust their sizes so that they were equal in area and approximately the same length and height. We turned the stimuli into videos with Adobe Flash for Macintosh, exported the videos as .mov files (30 frames per second), converted them to MPEG-4 for iPod in Apple iTunes, and presented them to the spiders on Apple iPods (generation 5; Apple Inc., Cupertino, CA). All trials were conducted in a room lit only by natural light filtered through a translucent blind; in pilot experiments, dim light improved spiders' attention to the videos.

Both pre- and post-training choice tests were run in a V-maze made of foam core (Fig. 2A). The end of each maze arm had a slot to accommodate an iPod. A hole cut through the bottom of the floor between the two arms allowed the spider to be inserted into the arena via a syringe. We coated the inside walls of the arena with Vaseline petroleum jelly (Unilever, Rotterdam, Netherlands) to keep spiders from escaping.

To begin a choice test, we placed a spider into an open-ended 30 ml syringe covered with opaque tape and plugged the syringe with a cotton ball wrapped in plastic wrap. We inserted the syringe into the bottom of the V-maze. We placed a V-shaped divider of clear acetate between the arms of the maze to prevent the spider from moving immediately into the maze upon release. After a 5-min rest, we removed the syringe plug and slowly depressed the plunger until it was flush with the floor of the maze. After the spider had clearly oriented to both stimuli (turning its body so that the anterior eyes were directed at the stimulus), the divider was removed and the spider was allowed to make a choice. A choice was defined as the spider walking 10 cm into an arm of the arena. If the spider did not orient to both stimuli at least once in 10 min before the divider was lifted or if it did not make a choice within 20 min after the divider

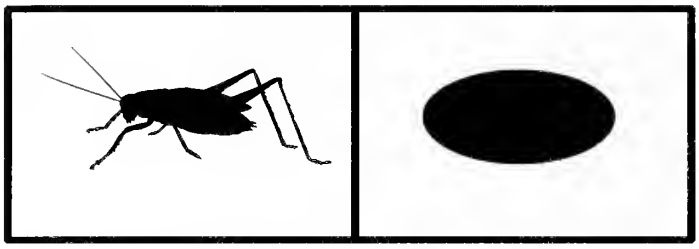

Figure 1.—The two stimuli used in the experiment.

was lifted, the trial was stopped and the spider was retested later that same day.

Spiders were trained in an alley constructed of foam core (15 cm × 9.5 cm × 11 cm high) (Fig. 2C, D). At one end of the alley, a slot held an iPod for playback of the training video. The distance between the spider and the iPod in the training arena was the same as the distance between the insertion point and the iPod in the choice testing arena. The opposite end of the alley had a 6.5 × 3.4 cm foam-core platform, separate from the floor of the arena and glued to the top of a 196 Hz 3V motor (Cermag Motor, Aristo-Craft, USA). An Arduino Uno (Smart Projects, Italy) was programmed to turn on the motor at specific intervals. We measured the vibration of the platform with an ADXL 335 3-axis accelerometer (Analog Devices, Norwood, MA, USA) driven by an Arduino Uno board. Raw data from the accelerometer was captured using CoolTerm (freeware.the-meier-s.org) at a sampling rate of 500 Hz and analyzed using the Data Analysis Tool Box in Excel (Microsoft Coporation, Redmond, WA, USA) and the Sinusoidal Motion Calculator (Vibration Calculator) from Advanced Mechanical Engineering Solutions (www.amesweb.info). The frequency spectrum for the motor showed a dominant peak at 196 Hz and a smaller peak at 190 Hz. The platform moved in all three axes of motion, with a peak displacement of 4.37 mm, 7.15 mm and 4.13 mm and a peak velocity of 68.67 mm/s, 112.38 mm/s and 64.87 mm/s along the x, y and z axes respectively.

For training with vibration, we tethered spiders in order to keep them oriented to a video of their preferred stimulus at a standard distance from the screen. We tethered each spider by waxing the tip of a microbrush (EasyinSmile Dental, Staten Island, NY) to the spider's cephalothorax as a "hat" (Fig. 2B). To attach the microbrush, we placed the spider in a 30 ml syringe with its tip cut off and a foam-padded plunger. We stretched a piece of Parafilm over the syringe opening and raised the plunger until the spider's cephalothorax and abdomen were firmly pressed against the Parafilm. We made a hole in the Parafilm to expose the cephalothorax while leaving the legs and abdomen immobilized. Using a heated wax carving tool typically used for the preparation of dental implants (GadgetWorkz, Orange County, CA), we melted several ml of a 1:1 beeswax/rosin mixture and dipped the microbrush into it. We then used a fine heated wax-carving tip to melt the mixture and attach the brush to the spider's cephalothorax while avoiding its eyes. We affixed hats at least 1 h before training.

During training, a spider was suspended by the end of its hat from an alligator clip attached to an adjustable XY microscope stage, which allowed us to easily orient the spider to the video. The spider was lowered onto the vibration platform until all of its tarsi made contact. We ensured that the cephalothorax and abdomen were clear of the platform because in pilot studies we had found that vibrating these disoriented the spider.

After the tethered spider was oriented to the video screen, it was given a three-minute rest with a blank screen followed by 10 training bouts (modified from Skow 2007). In each bout, the preferred stimulus appeared on the video screen and the spider was given 5 seconds of vibration every 10 sec for 30 sec. Each training bout was followed by a one-minute break during which the iPod displayed a

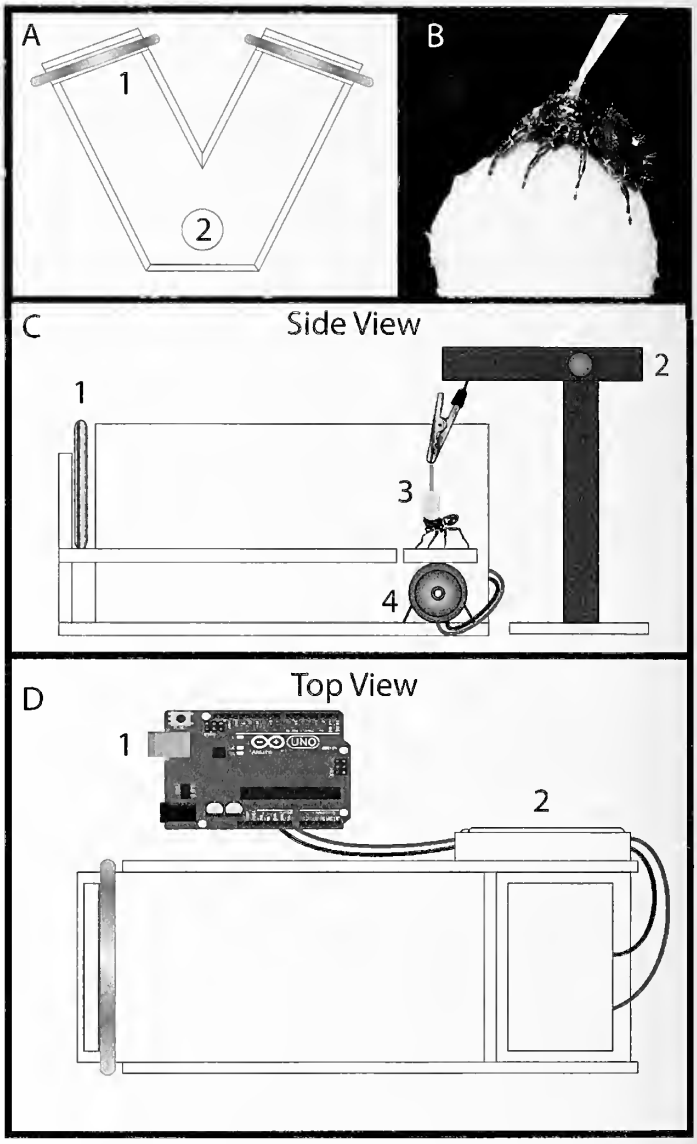

Figure 2.—A. Top view of the choice test arena. An iPod (1) was placed at the end of each arm, 12 cm from the center of the arena. A hole (2) was cut into the center of the arena to allow spiders to be introduced via a syringe with the end cut off. B. A spider tethered by a hat. C. Side view of the training arena. An iPod (1) was placed in an open slot at the far end of the arena opposite the spider. The spider was suspended over the vibration platform using a modified X/Y microscope stage (2) by a waxed hat (3). The vibration platform was glued to a small, 196 Hz motor (4), which provided the vibration. D. Top view of the training arena showing the placement of the Arduino Uno (1) and the battery case (2).

black screen. After the tenth training bout, we used forceps to gently pry off the spider's hat and repeated the choice test with the two stimuli randomly assigned to different arms of the choice arena. Control spiders underwent the same procedure except that the motor was disconnected from the vibration platform during training. If a spider did not make a choice after 20 min, the trial was ended and the spider was retrained and tested at least 24 h later. The vibration platform, syringe, plug and arena surfaces were cleaned with alcohol between trials.

In a separate test, we confirmed that spiders treated vibration as aversive by testing them in an arena (8.2 cm × 5.2 cm × 11 cm high) with half its floor comprised of the vibrating platform used in training

and the other half comprised of a stationary platform. The sides of the arena were covered in a thin layer of Vaseline to prevent escape. We tested two groups of spiders. For the vibration group ($n = 15$), a spider was placed in the arena, with the motor on the vibrating side turned off, and allowed to explore freely. After the spider had explored both sides of the arena floor and returned to the vibrating platform, we turned on the motor. We then recorded the amount of time the spider spent on the vibrating platform during a two min interval. The control group ($n = 15$) was treated identically but the vibrating platform was not turned on. We compared the amount of time each group spent on the vibrating platform with a Mann-Whitney U test.

While we saw no evidence during the learning experiment that spiders behaved abnormally after training, we also tested whether spiders stalked and fed on prey after being exposed to vibration. We followed the same hatting and training procedure described above except that we omitted the initial choice trial and showed the cricket video to all test spiders during training. After training, we removed the hats from the spiders, returned them to their cages, and allowed them to rest for 15 minutes. A single live cricket was then placed in each cage. We recorded the spiders' latency to attack and eat the prey.

Training with vibration was effective. Spiders appeared to be disturbed by the vibration of the platform and moved their legs rapidly in response (see Video 1, online at http://www.bioone.org/doi/suppl/10.1636/S14-49). Spiders exposed to vibration were significantly more likely than control spiders to choose the stimulus they did not see during training (Fisher exact probability test, $P < 0.04$). After vibration training, 7 of 30 spiders chose the stimulus that they saw during the training procedure, in contrast to 16 of 30 control spiders. In a separate test, freely moving spiders spent less time on a vibrating platform than did a control group where the vibration was turned off (Mann-Whitney U Test, $z = -3.69$, $P < 0.0002$). However, vibration did not appear to cause long-lasting harm, as all 15 spiders presented with live crickets after vibration training captured and ate the crickets within 15 min, and 13 of these did so within two minutes.

Two additional explanations for the training data should be considered. First, we cannot exclude the possibility that the tethering procedure itself was aversive and could cause spiders to avoid the stimulus they viewed during training; in fact, we view this as likely. However, the addition of vibration did significantly affect post-training decisions. Second, it is possible that vibration itself is not aversive to spiders, but that tethering with the wax hat is the aversive stimulus and that vibration only serves to increase the spiders' attention to it, similar to the role of vibration in VanderSal & Hebets (2007). However, freely moving spiders avoided a vibrating platform, supporting the hypothesis that vibration in itself is aversive.

Training with a 196 Hz vibration did not appear to cause long-lasting harm. However, we did not test other speeds. Our artificial vibration was in the range of seismic signals produeed by other jumping spiders. For example, *Habronattus dossenus* has a mating display with three seismic components with fundamental frequencies of 5.7–65 Hz and peak frequencies of 260–1203 Hz (Elias 2003). It is possible that faster vibrations would be harmful.

Our experiment demonstrates that vibration provides an alternative to another proven aversive stimulus, shock (e.g., Skow 2007; Bednarski et al. 2012). Shock has several drawbacks that vibration does not share. It is important to consistently apply the negative stimulus in order to ensure that all animals have the same opportunity for learning, but shock can be inconsistent. In a typical operant chamber, the animal receives an electric shock when it completes an open electrical circuit by touching two adjacent metal strips, rods, or parts of a grid. Larger animals such as rats or mice are in constant contact with both parts of the circuit, but we have noticed that spiders can learn to position their legs between even close strips and thus avoid shock. In addition, the amount of shock a spider receives varies depending on which part of its body completes the circuit. The amperage of the current passing through the animal depends on the electrical resistance of the animal's tissues. The resistance of chitin, which forms the spider's exoskeleton, increases with its thickness (Rao & Mehrotra 1997). Thus, the amount of shock a spider receives depends on whether the circuit is completed by the thick chitin of tarsal claws or by the thin chitin of the abdomen. In pilot data, we measured the current the spiders received in a shock chamber and found that it varied as they moved around. In contrast, vibration is visible so it easy to confirm that spiders are all receiving the same training.

Given the increasing interest in spider learning and cognition (reviewed in Jakob et al. 2011), vibration as a negative stimulus should be a useful addition to our tool kits. This training procedure could easily be modified in order to accommodate other experimental designs. While our trial used a tethered spider suspended over the vibration platform, the chamber could be modified to accommodate a free-running spider or could have a shuttlebox design.

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SHORT COMMUNICATION

Megaselia scalaris (Diptera: Phoridae): an opportunistic endoparasitoid of the endangered Mexican redrump tarantula, *Brachypelma vagans* (Araneae: Theraphosidae)

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Abstract. Despite the importance of tarantulas in the areas of medicine and veterinary science, there is very little information on parasitoid-tarantula interactions. The present study describes the case of an endangered tarantula, *Brachypelma vagans* Ausserer 1875, infested by an endoparasitoid in the field. Using DNA barcoding, we identified the parasitoid as the phorid *Megaselia scalaris*. With more than 500 fly larvae inside the host, this particular infestation can be considered severe. The size range of the larvae indicates infestation by all three larval instars. We discuss the possible mechanism by which the parasitoid is attracted to the tarantula and make important recommendations regarding improvements in tarantula-rearing conditions. Finally, this case study exemplifies the efficiency of molecular technology for parasitoid identification.

Keywords: Spider, parasitism, DNA barcoding, humpbacked flies, larvae morphology

Current knowledge on tarantula parasites and parasitoids is very limited. This is surprising considering the popularity of these spiders as pets and in zoos (Saul-Gershenz 1996), their use in medical (Park et al. 2008; Machkour-M'Rabet et al. 2011) and veterinary applications (Pizzi 2009), and that several tarantula species are protected. Consequently, any additional knowledge associated with tarantula parasites/parasitoids is relevant and indispensable.

Parasitoids are organisms characterised by first instars that grow on or inside the host and always kill it as part of their life cycle (Godfray & Shimada 1999), usually attacking different developmental stages of their host. Many species of spider are parasitized by a variety of insects (Eason et al. 1967), most of which belong to the arthropod orders Hymenoptera and Diptera (Korenko et al. 2011), as well as some nematodes (Poinar 1985, 1987; Penney & Bennett 2006), and kleptoparasitic spiders (Hénaut et al. 2005). Considering only dipteran parasitoids, those in the family Acroceridae are the most representative (Schlinger 1993), although some species from the Tachinidae, Chloropidae, and Drosophilidae families also parasitize spiders (Eason et al. 1967; Disney 1994). The Phoridae family comprises over 3000 species of small humpbacked flies found worldwide and includes scavengers, herbivores, predators, and parasites/parasitoids (Boehme et al. 2010). Parasitoid species of these flies are reported to parasitize mainly spider egg sacs. For example, larvae of *Phalacrotophora epeirae* Brues 1902, feed on the egg mass of spiders of various families (Muma & Stone 1971; Hieber 1992; Guarisco 2001). In addition, parasitoids of the genus *Megaselia* have been associated with numerous families of spiders including Araneidae (Finch 2005), Theridiidae and Lycosidae (Rollard 1990).

Tarantulas belong to the family Theraphosidae comprising 947 species (Platnick 2014). Although reports of tarantula parasitoids are extremely rare, the most recognized species is *Pepsis* spp. (Hymenoptera: Pompilidae) (Vardy 2000, 2005; Costa et al. 2004). Pizzi (2009) mentions that ichneumonid ectoparasites (Hymenoptera) possibly lay their eggs on captive tarantulas and also refers to two nematode families: Mermithidae and Panagrolaimidae, which parasitize wild and captive tarantulas respectively. Dermestid larvae (Coleoptera) parasitize captive *Brachypelma smithi* (Pickard-Cambridge 1897) specimens (Paré et al. 2001). Species of two Diptera families, Phoridae (Weinman & Disney 1997) and Acroceridae (von Eickstedt 1971, 1974; Cady et al. 1993), have also been reported as tarantula parasitoids.

Despite the high number of tarantula species in Mexico, only one study mentions the interaction between a parasitoid (*Pepsis* spp.) and a theraphosid spider (species of *Aphonopelma* Pocock 1901) (Punzo 2007). Of the 11 tarantula genera in Mexico (Platnick 2014), only *Brachypelma* Simon 1891 is protected under CITES (Appendix II). Throughout the last decade, efforts have been made to understand *Brachypelma* species and to contribute to their protection and conservation (e.g.: Machkour-M'Rabet et al. 2011, 2012; Vilchis-Nestor et al. 2013; Dor & Hénaut 2011, 2013; Dor et al. 2008, 2011).

A wild Mexican redrump tarantula, *Brachypelma vagans* Ausserer 1875 presented signs of weakness, leading to speculation that the spider was infested by fly larvae. After a short period of time, the tarantula died. No previous reports describe any manifestations or characteristics of a parasite infestation in this particular species of spider. Therefore, this occurrence presented a rare and exceptional opportunity to describe the case of an endoparasitoid infecting a protected species of tarantula.

The identification of a dipteran parasitoid, particularly as a larval instar, is problematical for the non-specialist taxonomist. DNA-based technology provides a possible solution to the problem of species identification. Hebert et al. (2004) developed an identification method known as "DNA barcoding", which uses part of the mitochondrial COI gene. This method is suitable for characterizing a large number of organisms (e.g.: Hebert et al. 2004; Prado et al. 2011), particularly parasitoids (Smith et al. 2007; Janzen et al. 2009; Zaldívar-Riverón et al. 2010), therefore, providing a unique opportunity to identify this specific tarantula parasitoid.

The aims of our study were i) to describe the manifestations presented by this spider during infestation and ii) to identify the endoparasitoid and describe the infestation.

The tarantula specimen was found in the village of "Laguna Guerrero" (Quintana Roo, Mexico) and taken to a laboratory maintained under standard conditions (25° C, 75% RH, natural light cycle). The tarantula was solitarily housed inside a plastic box (15 × 10 × 20 cm) to be reared for eventual reproduction. After a short period of time, the spider became inactive and showed no interest in food (adults of *Tenebrio molitor* Linnaeus 1758, Coleoptera: Tenebrionidae). Eventually, the tarantula stopped moving, as in pre-moulting behaviour, and its abdomen became abnormally distended. After two days, the tarantula adopted a huddled up position (all legs adducted, placing the tarsal tips under the sternum) and died. It was placed in 96% ethanol and after a few days, numerous dipterous larvae, assumed to have emerged from the spider, were observed in the alcohol (larvae deposited in the Zoological Museum of ECOSUR, Chetumal, Mexico).

All the larvae were collected from the alcohol and the tarantula was dissected to remove any remaining individuals from the carcass. The larvae were counted and their length measured (Stemi DV4 Zeiss stereomicroscope with measuring eyepiece, 32X magnification) to determine the larval instar. Twenty-five first and second-instar larvae were sent to the "Laboratorio de Microscopía Electrónica de Barrido" (Scanning Electron Microscopy Laboratory) at ECOSUR (Tapachula, Mexico) to confirm the presence of different larval instars and identify their morphological characteristics. Due to damage, third-instar larvae were not sent to the microscopy laboratory. Larvae were washed with 100% ethanol using a fine brush, submitted to several baths of 100% ethanol to remove any external elements and then dehydrated in 100% ethanol for 12 hours. They were subjected to critical point drying under CO_2 before being attached to double-sticky tape on aluminum stubs and coated with palladium-gold (20 nm thick) in a sputter-coating apparatus (Denton Vacuum, Desk II) for viewing under a scanning electron microscope (Topcon, SM-510).

For the molecular analysis by "DNA barcoding", five larvae were placed in a lysis 96-well plate with a drop of 96% ethanol. Genomic DNA was extracted from larval tissue and the extraction process was conducted following Montero-Pau et al. (2008). Amplification and sequencing of the DNA followed the protocols of Prado et al. (2011). Sequences and all collateral data from specimens are available on BOLD website (www.boldsystems.org) in the project entitled "PARTA".

Using the tools provided by BOLD-IDS, the obtained DNA barcode permitted identification to order and family level: Diptera and Phoridae respectively. The BLAST® tool from GenBank was then used for species level identification, providing a match with *Megaselia scalaris* Loew 1866 (99% similarity).

The *B. vagans* individual presented a high level of parasitism, hosting 524 larvae from a wide range of sizes representing the three larval-instars. The size frequency analysis suggests that second-instar individuals were dominant (Fig. 1). Following Sukontason et al. (2002) and Boonchu et al. (2004), the binomial distribution of size frequencies (Fig. 1) and the larvae ultrastructures (Fig. 2A–F) were used to determine the size range for each larval-instar. The size of second-instar larvae ranged from 1.0 mm to 3.5 mm (n = 466; 88.9% of total larvae), with a mean of 2.08 mm ± 0.02 (± SE) (Fig. 2A). The characteristic ultrastructures of the spiracular slits of the posterior abdominal spiracles (Fig. 2B) and the triangular-shaped labium, typical of second instar larvae, were identified (Fig. 2C). Some individuals were first-instar (from 0.5 mm to 0.9 mm; n = 55; 10.5% of total larvae) with a mean size of 0.65 mm ± 0.014 (Fig. 2D). These larvae showed rudimentary posterior abdominal spiracles that presented a broad-based posterior spiracular hair (Fig. 2E) and a characteristic bi-lobed labium (Fig. 2F). There were only three third-instar individuals (from 3.6 mm to 3.8 mm; n = 3; 0.6% of total larvae) with a mean size of 3.7 mm ± 0.058. As these larvae were damaged, no morphological characteristics were identified.

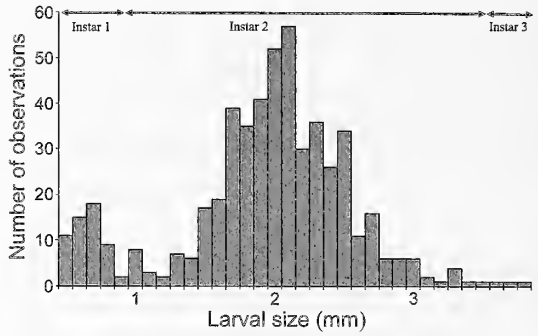

Figure 1.—Frequency of larval sizes (mm) for the endoparasitoid *Megaselia scalaris* (Diptera: Phoridae) taken from a specimen of *Brachypelma vagans* (Araneae: Theraphosidae).

Megaselia scalaris is a cosmopolitan phorid fly with larvae that feed on a high diversity of decaying organic material, making this species a facultative predator, parasite, and parasitoid in invertebrate laboratory colonies (Costa et al. 2007; Disney 2008).

Megaselia is known to parasitize theraphosid spiders in Colombia (Weinmann & Disney 1997) and spiders of the genus *Theraphosa* Thorell 1870 in French Guiana (Marshall & Uetz 1990). However, this is the first report of a living endangered Mexican tarantula species hosting a parasitoid in the wild. Although no observations were made, *M. scalaris* adults were probably attracted by the accumulated remains (prey and moult) in the tarantula burrow (Machkour-M'Rabet et al. 2007). These flies became parasitoids of the living spider by using the book lung as an entrance point and subsequently penetrating the opisthosoma and internal organs (Pizzi 2009). This hypothesis is substantiated by several studies that describe phorid adults feeding on the spider's prey (Sivinski & Stowe 1980; Weinmann & Disney 1997) and being attracted to the stabilimentum of the spider's web by the strong smell of decaying matter (Hénaut et al. 2010). Weinmann & Disney (1997) reported the presence of phorid larvae on living specimens of two theraphosid species, *Megaphobema robustum* Ausserer 1875 and *Pamphobeteus* Pocock 1901, in Colombia. The phorid species were identified as *Megaselia dimorphica* Disney 1997 and *Megaselia praedafura* Disney 1997. Marshall (pers. obs. in Marshall & Uetz 1990) reported a *Megaselia* fly associated with *Theraphosa* spiders in French Guiana. In another study, Pérez-Miles et al. (2005) suggest that the silk that covers the burrow entrance during the day provides protection against parasitoids.

The tarantula's death was not unexpected as the level of infestation, (over 500 larvae) was considered very high, One study reports 138 specimens of *M. scalaris* on a piece of sardine (Moretti et al. 2009). This number of larvae is not exceptional when considering the high fecundity of *M. scalaris* females that can lay up to 600 eggs (references in Disney 2008). The high level of infestation by different fly instars could be the result of a single female oviposition over a period of several weeks, or ovipositions from different females at different times.

Parasitoidism by phorid flies poses a potential risk to tarantula breeding for pets or scientific use. Therefore, it is crucial that these spiders are adequately managed and protected. Constant cleaning, maintaining optimal temperature and humidity, control of new individuals through a quarantine period, and the mechanical protection of spiders from parasitoid arrival would substantially reduce the risk of infestation by this dipteran on *Brachypelma* spp. Furthermore, the identification of a parasitized *B. vagans* in the field highlights the potential risk for natural populations of these endangered tarantulas. More research is necessary to evaluate the impact of fly parasitoids on wild tarantula populations

Megaselia scalaris was successfully identified using DNA barcoding. Because morphological determination to the species level is

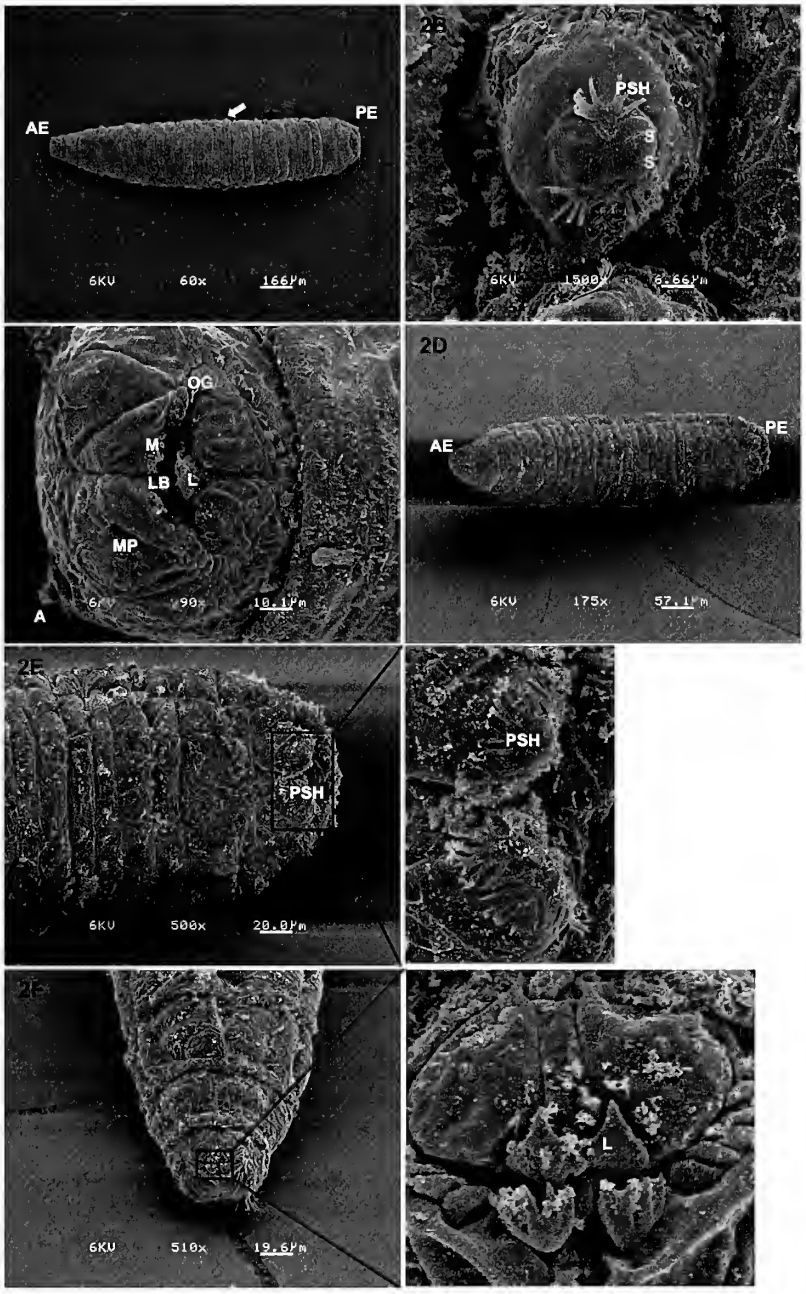

Figure 2.—Scanning electron micrographs of *Megaselia scalaris* (Diptera:Phoridae) larvae taken from a specimen of *Brachypelma vagans* (Araneae: Theraphosidae). (A) Ventral view of the entire body of a second-instar larva with anterior end (AE) and posterior end (PE). White arrow indicates short spinous process and black arrow shows cephalic segment. (B) Posterior spiracular disc of a second instar larva with its two straight slits (S) for each expanded end, and the posterior spiracular hairs (PSH). (C) Frontal view of the cephalic segment of a second-instar, illustrating the antenna (A), labium (L), labrum (LB), oral groove (OG), mouth hooks (MH) and maxillary palp complex (MPC). (D) Ventral view of the entire body of a first-instar with anterior end (AE) and posterior end (PE). (E) Broad-based posterior spiracular hairs of a first-instar (PSH). (F) Frontal view of the cephalic segment illustrating the bi-lobed labium (L) of a first-instar.

especially difficult for larvae and pupae, the use of a DNA-based method is an excellent alternative. We hope that this DNA barcoding technique will become a straightforward laboratory routine for non-specialists in molecular ecology in order to rapidly resolve issues of specimen identification.

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General: The *Journal of Arachnology* publishes scientific articles reporting novel and significant observations and data regarding any aspect of the biology of arachnid groups. Articles must be scientifically rigorous and report substantially new information. Submissions that are overly narrow in focus (e.g., local faunal lists, descriptions of a second sex or of a single species without additional discussion of the significance of this information), have poorly substantiated observational data, or that present no new information will not be considered. Book reviews will not be published.

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Title page.—The title page includes the complete name, address, and telephone number of the corresponding author; the title in sentence case; each author's name and address; and the running head.

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